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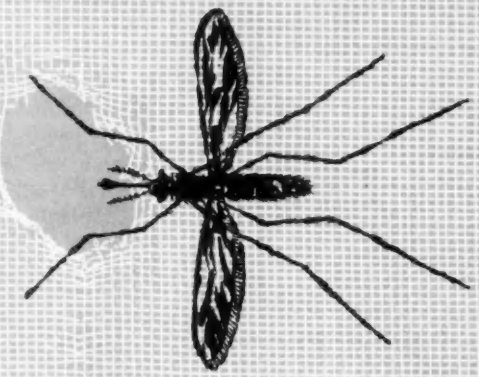

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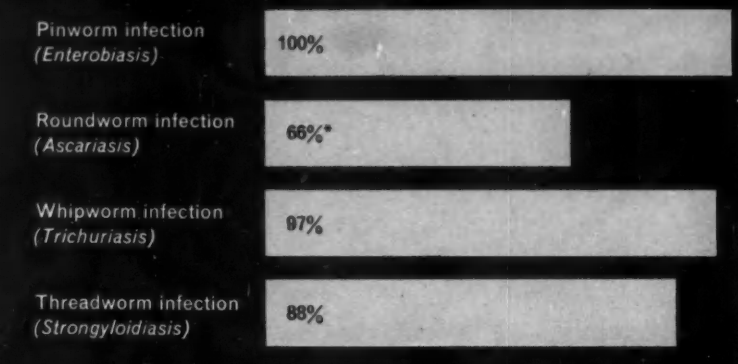

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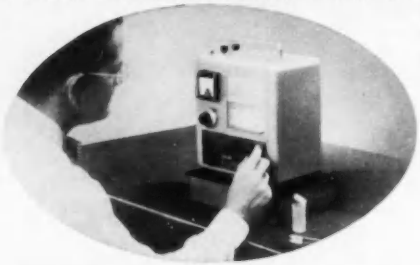

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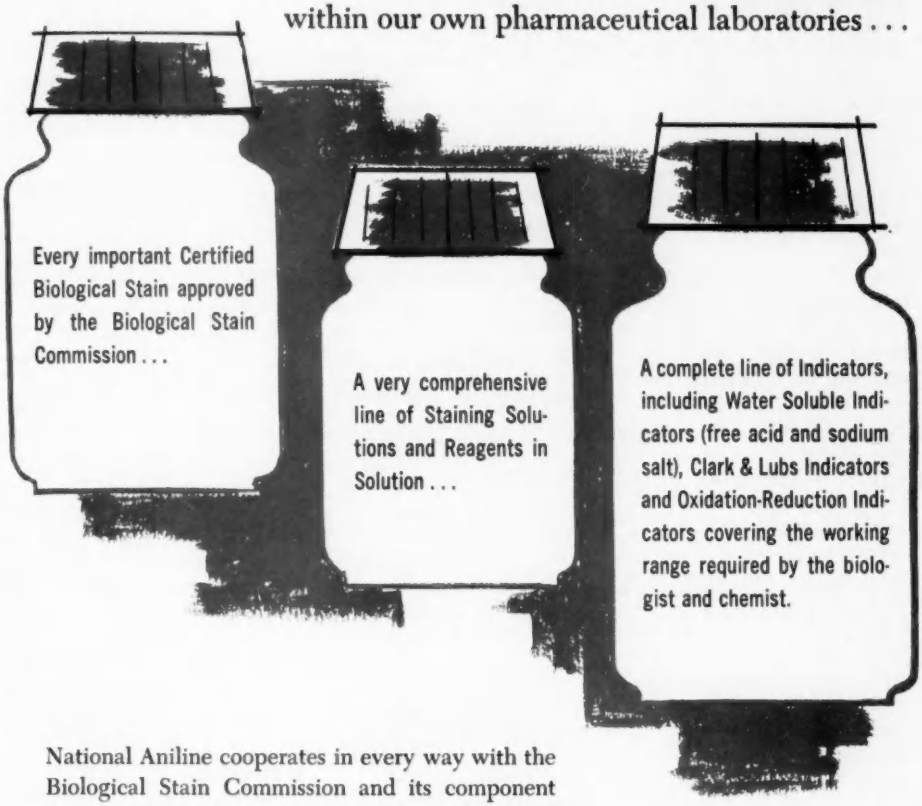
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PARASITOLOGIC SURVEYS IN CALI, DEPARTAMENTO DEL VALLE, COLOMBIA. V. CAPACITY OF *ENTAMOEBA HISTOLYTICA* OF HUMAN ORIGIN TO UTILIZE DIFFERENT TYPES OF STARCHES IN ITS METABOLISM

ERNEST CARROLL FAUST* AND THOMAS R. READ†

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INTRODUCTION

Probably the most useful single step contributing to the elucidation of the natural history of *Entamoeba histolytica* consisted in the development of technics for its in vitro cultivation. Originally designed to aid in the diagnosis of this ameba when microscopic examination of human stools had been unrewarding, cultivation of the organism has served a much more extensive use in providing material for life history studies, for in vivo inoculation investigations, and for biochemical and immunologic inquiries. In the culture tube, just as in the large intestine of man and susceptible experimental hosts, colonization of *E. histolytica* depends on a suitable environment.

The first reproducible method for in vitro culture of *E. histolytica* was that of Boeck and Drbohlav,[5] who devised two diphasic media, Locke-Egg-Serum (LES) and Locke-Egg-Albumin (LEA), both without starch. Craig[8] utilized a simplified monophasic medium consisting of inactivated serum in Locke's solution, also without a starch substrate. Beginning with the culture method of Dobell and Laidlaw[9] and with only one notable exception,[22] practically all other workers who have developed new culture technics or have modified the LES and LEA media have introduced starch into the culture tubes in which the ameba is planted.[2, 7, 10, 19, 21, 23, 24, 25] St. John[21] added whole-wheat flour to his heart-muscle broth culture; all others utilized rice starch or rice flour. Dobell and Laidlaw[9] tested starches of rye, maize, buckwheat and rice, and concluded that rice starch was preferable because of its smaller size. Since that time rice starch has become a standard substrate in cultures of *E. histolytica* to furnish energy for luxuriant growth. Recently Benham and Havens[4] have found starch grains of *Amaranthus paniculatus*, *Kochia scoparia* and *Mirabilis jallapa*, with particle size 2 μ, 0.5–1.0 μ and 1–2 μ, respectively, to be good substitutes for rice starch. Hilker *et al.*,[17] have pointed out that it is apparently not particle size alone which is important in rice starch but also its molecular configuration which determines the ability of *E. histolytica* to convert it into sugars.

The study on which this report is based has had a somewhat different objective from those of earlier investigations, namely to test in vitro the capacity of *E. histolytica* to ingest and utilize several starches which constitute the principal food of the economically lower class of ward Siloé, Cali, Colombia, a city of half a million population, which is situated in tropical northwestern South America. Microscopic direct and zinc sulfate concentrates of fresh fecal specimens from about 1,000 persons belonging to 100 families in ward Siloé, have revealed approximately 40% infection with *E. histolytica*. Although 80% of these amebae belong to the large race, with mean cyst diameters measuring 10 μ or more, rarely is there evidence of clinical amebiasis.[11, 12] Moreover, in practically all diagnosed cases, only cysts of *E. histolytica* have been recovered from the stools, which are formed or semiformed.

In a parasitologic survey of this population undertaken in November, 1956, microscopic preparations of the feces have commonly revealed an appreciable amount of undigested starch grains in the stools. This suggested that the availability of starch in transit through the large intestine of members of this population might serve as food for *E. histolytica*, thus providing a satisfactory milieu for its colonization without need for its invasion of the intestinal wall to maintain its existence.

* Field Coordinator, Tulane-Colombia Program in Medical Education, International Cooperation Administration, Mutual Security Program of the United States Operational Mission to Colombia.

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MATERIALS AND METHODS

The following starches have been tested: rice powder furnished by Professor Paul C. Beaver of Tulane University, New Orleans, Louisiana, and plantain, yucca, corn, Irish potato, white sweet potato and white wheat starches obtained locally in Cali, Colombia. The study conducted in Colombia utilized Balamuth's culture medium, and transfers were made every 48 hours. Cali strain no. 1523 (large race) of *E. histolytica* with its associated bacteria was used in all tests conducted in Colombia. This strain was isolated June 17, 1957, by Dr. Iris M. Krupp from an asymptomatic carrier and was one of 14 which were inoculated into guinea pigs to test their pathogenicity.[15] Following several transfers and luxuriant multiplication with rice starch as a substrate, transplants were made to the same culture medium containing the substituted starches, in each series with control cultures containing rice starch. Although a minimal amount of rice starch was carried over in decreasing amounts in the first few subcultures provided with the substituted starches, by the fourth or fifth subculture only the latter were present. Subsequently, in the Fall of 1957, one of us (TRR) tested the capacity of two non-Colombian strains of *E. histolytica* (no. "200," and no. "H" of Beaver *et al.*,[3] to utilize yucca and Irish potato starch from the same Colombian supply. Meanwhile one of us (ECF) had studied the relative sizes and morphology of the seven starches tested and was able to identify both intact and partially digested starch grains of plantain and yucca passed in the stools of persons whose feces had been examined and had been found to contain cysts of *E. histolytica*.

PRESENTATION OF DATA

The principal experiments to be reported were begun in June and concluded early in September, 1957. Counting the pretesting period, *E. histolytica* with rice starch was carried through 40 subcultures. Those with wheat flour, initiated July 9, survived through 29 subcultures; those with yucca starch, started July 10, through 28 subcultures; those with plantain, Irish potato and white sweet potato, begun July 23, continued through 22 subcultures, in all instances until the tests were terminated. In cultures with corn starch, *E. histolytica* died after the 16th transfer. Although there were periods when all of the cultures, including those with rice starch, showed poor growth, there was continued evidence that the amebae were able to ingest all of the substituted starches, even when the starch grains were as large as the ameba trophozoites; and, with the exception of corn-starch cultures, the amebae were able to propagate satisfactorily through one subculture to the next in the absence of rice starch.

Our observations on gradual size reduction and surface digestion of the ingested substituted starches and our positive test for glycogen-like substance within the trophozoites' endoplasm are consistent with the biochemical analyses of Hallman and DeLamater[15] and of Baerstein *et al.*,[1] who demonstrated amylase production by the ameba, and of Hilker *et al.*,[17] who showed the presence of amylase and maltase in lyophylized preparations of *E. histolytica* trophozoites.

In Plate I camera lucida sketches drawn to scale illustrate unmodified grains of the seven types of starch utilized in the present study (*1–7*), and starch grains, intact or partially digested, recovered from six representative stools from which *E. histolytica* cysts had been diagnosed (*8–13*). A majority of the latter are diagnosable as plantain starch grains (Pl. I, *1*), whereas, in at least one instance (Pl. I, *10*), they resemble those of yucca (Pl. I, *2*). Plantain and yucca are the principal constituents of the food consumed by the population under study.

The surface of plantain starch grains (Pl. I, *1*) reminds one of the shells of several species of clams and mussels, with laminations originating near one pole and continuing to the opposite pole; they are typically broader at one end and are circular in cross-section. These grains vary in size from about $11 \times 7\ \mu$ to $38 \times 25\ \mu$ and $42 \times 15\ \mu$. While several were occasionally seen within a cell membrane, they were usually separate in the dehydrated material which was specially prepared for us from green plantains. In the unripe boiled state in which they are consumed by the Siloé population, a considerable amount of these starch grains remains undigested and appears in the stools, recognizable as plantain starch (Pl. I, *8*, *9*, *11*).

The grains of yucca starch (Pl. I, *2*) are equally distinctive. They are spherical or subspherical, with a deep stellate or lenticular fissure on one side. Some of the grains have superficial surface

fractures, suggesting that they scale off rather easily. The grains measure from 9.5 to 27 μ in diameter; those about 13 μ have been seen in considerable quantity in some stools (cf. Pl. I, *10*, possibly *13*).

Maize starch (Pl. I, *3*), obtained locally as a commercial product, most frequently resembles a 5-sided truncated pyramid of variable but low height, usually with a minute central depression. The sides may or may not be equilateral; at times the base is nearly circular. The grains vary from 12 to 21 μ in breadth at their base. No recognizable maize starch grains have been seen in more than 3,000 stools examined from this population; this is probably due to the fact that corn is not a major item in the diet of these individuals, and not necessarily to its easy digestion.

Irish potato starch (Pl. I, *4*), locally prepared for us, is characteristically broadly ovoidal and is equally broadly rounded at the ends. It possesses superficial laminations as does plantain starch (Pl. I, *1*) but, in addition, frequently has a punctate, lenticular, cruciform or stellate depression at the pole where the laminations terminate. The grains vary remarkably in size, from 13.5 $\times$ 11.5 μ to 86.5 $\times$ 57.5 μ. These starch grains have not been seen in the stools.

White sweet potato starch (Pl. I, *5*), which was specially prepared for us, has a variety of configurations. The individual grains may be spherical, broadly ovoidal with a depression at one end, and may be twinned or massed in groups of threes. Every grain studied had a trident, cruciform or stellate depression at one end, but lacked surface laminations. The grains measure 13.5 to 25 μ in diameter when spherical, and up to 36 $\times$ 25 μ when ovoidal. It is not surprising that this type of starch has not been found in the stools since this root crop is seldom eaten by the Cali population although it is found in the markets.

The starch grains in local wheat flour (Pl. I, *6*) are the most distinctive of the seven types studied. They superficially remind one of a coffee bean, with smooth contour and, on one face, a long cleft which may be forked at one or both ends. The grains measure 16 $\times$ 7.5 μ to 23 $\times$ 13.5 μ. Although flour in bread, or more commonly in macaroni, is often consumed as a minor item of diet by the population in our survey, no flour starch has been seen in their stools. It may have been digested or have been hydrolyzed by the ameba.

Rice starch (Pl. I, *7*), in general, resembles corn starch in its architecture, namely in having the form of a truncated pyramid. However, the grains are invariably smaller, at times are still fitted together in polygonal groups one or more layers deep, and usually are 6-sided at their bases. In the rice flour furnished by Dr. Paul C. Beaver, the grains measured from 4 to 9 μ in greatest cross section; Difco rice powder also had starch grains varying from 4 to 9 μ, with 7 μ as the most frequent particle size. Recognizable rice grains have not been seen in the stools of the local population; they may have been digested or utilized by the ameba.

Plate II provides free-hand sketches, drawn to scale, of the contour and movements of *E. histolytica* trophozoites with ingested starch grains of the seven different types during 30-minute observation periods (except for a 20-minute period for *6a* and a single representative of *7a*. In each of the seven series Figure *a* is drawn from an early subculture and Figure *b* from a later one of the same substrain.

1. *Plaintain starch.* Figure *a* (3rd subculture) shows slight decrease in size of a single, large, ingested starch grain; trophozoite 24.7 μ and starch grain 16.5 $\times$ 11 μ in size at the end of the period. Figure *b* (20th subculture) has a single grain which has apparently split in two; these were then extruded and another grain ingested during the observation period; trophozoite 41.3 μ in length and newly ingested starch grain 13.7 μ in length at the end of the period.

2. *Yucca starch.* Figure *a* (7th subculture) shows a single characteristic grain in the endoplasm; trophozoite 55 μ long and starch grain 12.4 $\times$ 8.3 μ at the end of the period. Figure *b* (24th subculture) trophozoite 24.7 μ and two starch grains, one extruded and the other measuring 8.3 $\times$ 5.5 μ at the end of the period.

3. *Corn starch.* At the beginning of observation Figure *a* (4th subculture) contained three characteristic corn starch grains, two of which were gradually decreasing in size but all three were extruded at the end of 30 minutes; trophozoite near the end of observation 44 μ in length; largest starch grain 13.7 $\times$ 11 μ. Figure *b* (16th subculture) represents a culture containing both corn starch and rice powder, which produced much better growth of the amebae than corn starch alone, with evidence of much more rapid utilization of the rice starch; trophozoite 41.3 μ

and corn starch grain 11 × 9.6 μ at end of observational period.

4. *Irish potato starch.* In both instances the ameba had ingested a single large starch grain which was gradually being digested. At end of the period Figure *a* (8th subculture) trophozoite measured 30.2 μ in greater diameter and ingested starch grain 13.7 × 11 μ; Figure *b* (21st subculture) trophozoite measured 38.5 μ in length and ingested starch grain 16.5 × 13.1 μ.

5. *White sweet potato starch.* Figure *a* (11th subculture) and Figure *b* (22nd subculture) both represent an ameba that had ingested a single large starch grain which became slightly smaller during the observation period. At the end of the period Figure *a* trophozoite measured 40 μ in length and the ingested starch grain 11 × 8 μ; Figure *b* trophozoite measured 38.5 μ in length and the ingested starch grain 11 × 6.8 μ.

6. *Flour starch.* During 20 minutes of observation of the trophozoite in Figure *a* (4th subculture) there was no evidence of digestion of the single starch grain (15.1 × 13 μ) which was almost as large as the ameba (16.5 μ); the trophozoite of Figure *b* (26th subculture) was very much larger (52.2 μ) and the single starch grain 13.7 × 6.9 μ.

7. *Rice starch.* Observation was made in March, 1957, on a flourishing culture of large race *E. histolytica*; Figure *a* a single, very active trophozoite with 10 starch grain inclusions, from seventh subculture; Figure *b* from 27th subculture, with nine ingested starch grains rapidly undergoing hydrolysis; Figure *a* trophozoite about 50 μ in length and starch grains about 7 μ in breadth; Figure *b* trophozoite at end of observation 40 × 32 μ, starch grains almost completely digested within food vacuoles.

In New Orleans during the late Fall of 1957 one of us (TRR) tested Colombian yucca and Irish potato starches as substituted substrates for in vitro cultivation of two non-Colombian strains of *E. histolytica*, "200" and "H" of Beaver *et al.*,[3] employing modified LES diphasic medium. Strain "200" was carried successfully through 16 48-hour subcultures; the "H" strain began to grow vigorously but died after a few transfers due to undetermined causes. Both strains avidly ingested both the yucca and potato starches. Thus, there is evidence that the acceptability of these starches as a source of carbohydrate is not limited to the Colombian strain of *E. histolytica* employed in our earlier tests.

DISCUSSION

The seven starches tested by us for in vitro utilization by *Entamoeba histolytica* are produced by plants belonging to five different families, viz., rice (*Oryza sativa*), maize (*Zea mays*) and wheat (*Triticum aestivum*), Family Gramineae; Irish potato (*Solanum tuberosum*), Family Solanaceae; white sweet potato (*Ipomea batatas*), Family Convolvulaceae; plantain (*Musa paradaisica*), Family Musaceae, and yucca (*Yucca gloriosa*), Family Amaryllidaceae. The structural characteristics and size ranges of the respective starches have been described and illustrated (Pl. I, above). Rice provides the basic diet on the north coast of Colombia, maize in the mountain departments of Antioquia and Caldas and in the Indian populations in other areas; Irish potatoes and wheat primarily in the high plateau region around Bogotá, and plantain and yucca in the department of Valle, of which Cali is the capital. Sweet potatoes are of minor importance in the diet. According to the Instituto Nacional de Nutrición, Bogotá (1953), the calories in 100 grams of each of these foods are as follows: rice 359; maize (grits), 348; peeled Irish potatoes 91; wheat (macaroni), 350; (unfortified bread) 301; green plantain, 140; yucca tubers, 146; and peeled sweet potatoes, 89. A preponderance of the calories in these foods is derived from the starch contents, with low protein except for maize grits and wheat, low fat except for the whole-kernel yellow corn and wheat, low iron except for these same products, no vitamin A in rice, maize grits, wheat and Irish potatoes, but high vitamin A in yellow corn, sweet potatoes and plantain, low thiamin, riboflavin and niacin, and no ascorbic acid in polished rice, maize grits and unfortified wheat bread, but relatively high in Irish and sweet potatoes, plantain and yucca. In a recent (unpublished) preliminary study of ten representative families consisting of 132 individuals in the Family Care Program of the Department of Preventive Medicine and Public Health, University of Valle (the same group in which parasitologic surveys are being carried out), the average daily nutrients in the food consumed are indicated in Table 1, column 2, with comparison (column 3) of the recom-

mended daily amounts of the Food and Nutrition Board, U. S. National Research Council.[13]

Examination of Table 1 shows average deficiencies in the diets of the Siloé population as follows; calories, 2- to 4.5-fold, resulting from insufficient intake of carbohydrates and fats; proteins, 2.5- to 4-fold, and in all the essential minerals and vitamins except ascorbic acid, which is supplied from plantain and yucca. Fats which are consumed are derived almost entirely from vegetable shortening, not from meats and egg yolk. Proteins, likewise, are principally of vegetable origin, with very meager supplements of beef, and milk (for the youngest weaned child).

In diets deficient in protein, even if adequate in calories (which the data in Table 1 indicate is not the case), "secretion of the pancreatic enzymes amylase, lipase and trypsin is reduced"[6] (p. 963) while "intestinal enzymes are also likely to be involved."[20] This insufficiency of pancreatic amylase and intestinal maltase can explain the failure to split the starches of plantain and yucca to maltose and glucose, so that the value of intake of calorie-producing carbohydrates, insufficient as it is, is still further reduced. As a result of deficiency in amylase and maltase production undigested starch grains are often found in the stools of persons in this area. Thus, the relatively high ratio of carbohydrate in the form of undigested starch to protein intake appears to have a direct relationship to the availability of these starches for *E. histolytica* trophozoites in the large intestine.

The present study has demonstrated that plantain, yucca and other starches consumed by the Siloé population are acceptable for metabolic needs of *E. histolytica* in culture, even though these starch grains are much larger than those of rice. In a personal communication[16] Dr. Doris M. Hilker, who has kindly analyzed the starches which we have tested, states that she has found rice and Irish potato starches to contain relatively large amounts of amylopectin (branched-chain component), that maize starch has a preponderance of amylose (straight-chain component), while plantain and yucca starches are intermediate in their configuration. Wheat and white sweet potato starch are composed principally of amylose, although extraneous insoluble material in the samples analyzed, not present in those of the other starches, may possibly have prevented

TABLE 1

Comparison of average daily nutrient intake per person in ten families, Ward Siloé, Cali, Colombia, compared with recommended diets of Food and Nutrition Board, U. S. National Research Council (1953)

Category	Ward Siloé, Cali	N.R.C. (U.S.) (Persons over 6 yrs. of age)
Calories	1,130	2,000–4,500
Carbohydrates (g)	225.1	Normally supplies 50% of calories*
Proteins (g)	24.4	60–100
Fats (g)	21.8	Normally supplies 35% of calories†
Ca (mg)	182.3	1,000–1,500
Fe (mg)	6.1	10–15
P (mg)	480.2	1,500‡
Vitamin A (Int'l units)	1,426.0	2,500–6,000
Thiamin (mg)	0.4	1.0–1.8
Niacin (mg)	5.9	8.0–18.0
Riboflavin (mg)	0.4	1.5–2.5
Vitamin C (mg)	61.1	60–100
Vitamin D (Int'l units)	—	400

* Nelson, Waldo E., 1955. Textbook of Pediatrics, p. 80. Phila. and London, W. B. Saunders & Co., 1581 pp.

† Ibid., p. 81.

‡ Ibid., p. 85.

detection of minimal amounts of amylopectin. Hilker *et al.*,[17] have previously demonstrated that *E. histolytica* trophozoites contain amylase and maltase, providing the starch-hydrolyzing enzymes elaborated in the human pancreas and small intestine. In ward Siloé population, with its 40% *E. histolytica* infection and protein deficient diet, the ameba is potentially able to utilize the starches which the host is unable to digest. Possibly under these particular circumstances this enzyme activity of *E. histolytica*, with an abundance of usable starch in its immediate environment to meet its metabolic requirements, safeguards the host, since it usually appears to prevent amebic invasion, or at least deep amebic invasion of the intestinal wall, thus effecting a relatively satisfactory equilibrium between host and parasite. Although similar conditions undoubtedly exist in many other parts of the world where asymptomatic or "carrier" states are being discovered by clinicians, we are not aware that this explanation of causal rela-

tionship has been previously offered for this symbiotic type of existence between a demonstrated potentially pathogenic *E. histolytica* and asymptomatic infection in the human host.

Our comparative tests with the seven starches have been made with large-race strains of *E. histolytica*. With them we have been able to confirm the view of Hilker *et al.*,[17] that particle size "apparently is not the only factor involved," and that amylase secreted by the ameba plays an important role in starch utilization by the ameba. Yet small-size starch grains composed principally of branched molecules are probably more satisfactory even for large-race strains of the ameba and, if one considers that small-race trophozoites are at times smaller than rice-starch grains, an explanation may be provided for the difficulty encountered in getting small-races of *E. histolytica* into culture.

There is a suggestion in our in vitro studies that, in the later subcultures of *E. histolytica* after the ameba had become adapted to the substituted starches, there was an increase in size of the trophozoites compared with earlier subcultures. This was the case with plantain (Pl. II, *1 b* vs. *a*), Irish potato (Pl. II, *4 b* vs. *a*), and particularly flour starch (Pl. II, *6*, *b* vs. *a*). However, one strain with rice starch substrate (Pl. II, *7*) grown through 32 subcultures, with frequent encystation, demonstrated marked size range of trophozoites from one subculture to another, varying from 6.8 to 40 μ, and with mean cyst size from 10.4 to 15.4 μ, but more frequently 13.4 μ. This remarkable size variation, especially of the trophozoites, may be due to any one of several factors or a combination of factors within the culture tube and, by inference, within the host's large intestine, including abundance or paucity of helpful or harmful bacterial associates. These micro-environmental influences which temporarily affect the size of the amebic trophozoite may, in turn, determine the ability or inability of the ameba to ingest large starch particles. Thus, size of the trophozoite at a particular time in its cycle does not in itself provide evidence of its average size. The usual mean cyst size is a much more dependable index.

SUMMARY AND CONCLUSIONS

1. Forty % of 1,000 persons in 100 representative families of an underprivileged population group in ward Siloé, Cali, Colombia harbor strains of *Entamoeba histolytica*, 80% of which belong to the large race; yet clinical manifestations of this infection are rare. The principal food of these persons consists of starches, derived mostly from cooked green plantains and yucca tubers, less commonly macaroni, unfortified bread, Irish potatoes, corn grits, rice and, rarely, white sweet potatoes. Protein and fat supplements, mostly from plant products, are consumed in meager supply and, except for vitamin A in plantains and yucca, vitamins are very inadequate in the diet, while essential minerals are deficient.

2. Because undigested starch grains, identified as those of plantain and at times yucca, are frequently evacuated in the stools of these individuals, it seemed desirable to investigate the capacity of *E. histolytica* to ingest and utilize the starches consumed by the population to serve the metabolic needs of the parasite.

3. A large race *E. histolytica*, isolated from human feces for pathogenic studies in guinea pigs, was first cultured in vitro with rice starch in Balamuth's monophasic medium. Following a number of subcultures, starches obtained from plantain, yucca, maize, Irish potato, white sweet potato and wheat were substituted for the rice starch, which served as control in parallel tubes. Evidence of satisfactory utilization of these substituted starches was obtained for plantain, Irish potato and white sweet potato (22 subcultures), yucca (28 subcultures) and wheat (29 subcultures), each continued until the experiments were terminated; in contrast, trophozoites in the maize starch died out after the 16th subculture. In addition, two non-Colombian strains of large-race *E. histolytica* ("200" and "H") were later tested in New Orleans, employing the same Colombian yucca and Irish potato starches in modified LES medium. Both strains started to grow vigorously with the substituted starches; "200" continued through 16 subcultures but "H" died after a few transfers.

4. The experiments demonstrated the ability of a Colombian strain of *E. histolytica* to propagate satisfactorily in vitro when plantain, yucca, Irish protato, white sweet potato and wheat starches were substituted for rice starch in the culture medium, partial success with corn starch, and for yucca and Irish potato starches to serve the metabolic needs of one non-Colombian strain ("200") through numerous transfers.

5. In considering the culture tests with respect to the infected population, controlled nutritional studies in many parts of the world indicate that inadequate protein intake such as exists in our population provokes deficient pancreatic and intestinal enzyme production (*e.g.*, amylase and maltase), thus probably explaining the presence of plantain and yucca starch grains in the stools of these persons.

6. Biochemical studies have demonstrated that *E. histolytica* elaborates amylase and maltase, capable of splitting ingested starch grains and converting them into sugars. The degree of utilization depends on whether the starches contain large amounts of amylopectin (branched-chain component, most easily hydrolyzed by the ameba) or principally amylose (straight-chain component, more resistant to hydrolysis by the ameba). Rice and Irish potato starches consist mostly of the former component, maize, wheat and sweet potato starches of the latter, while the other two starches tested are intermediate in their molecular configuration.

7. Relating the tests carried out in this study with nutritional investigations and the biochemical analyses, it seems permissible to consider the sequence of events as follows: (a) protein malnutrition produces pancreatic and intestinal enzyme deficiency so that ingested starches are inadequately hydrolyzed, with consequent passage of intact starch grains through the intestine and their evacuation in the stools; (b) *E. histolytica* trophozoites colonizing in the large intestine utilize certain of these starches for their metabolic needs and thus are usually able to maintain their colonies in the intestinal crypts without deep invasion of the intestinal wall; (c) this last link in the chain may explain why the infected population harbors *E. histolytica* as an essentially asymptomatic infection, even though collateral studies have demonstrated that large race strains of this ameba in this geographic area are highly pathogenic.

8. Although biochemical tests and our studies indicate that size of the starch grains is not the only factor concerned with the ameba's ability to utilize intact starch grains present in its immediate environment, if trophozoites belonging to a small race are physically incapable of ingesting the starch grains, a plausible explanation is provided for the difficulty frequently experienced in getting small races into culture, even when the starch is of a demonstrably utilizable type.

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Plate I. Camera lucida sketches, all drawn to same magnification, of seven types of starch grains tested in in vitro studies with *Entamoeba histolytica*. *1–7*, from samples tested: *1*, plantain; *2*, yucca; *3*, maize; *4*, Irish potato; *5*, white sweet potato; *6*, wheat; *7*, rice; *8–13*, undigested and partly digested starch grains recovered from feces of *E. histolytica*—infected persons in population of ward Siloé, Cali, Colombia.

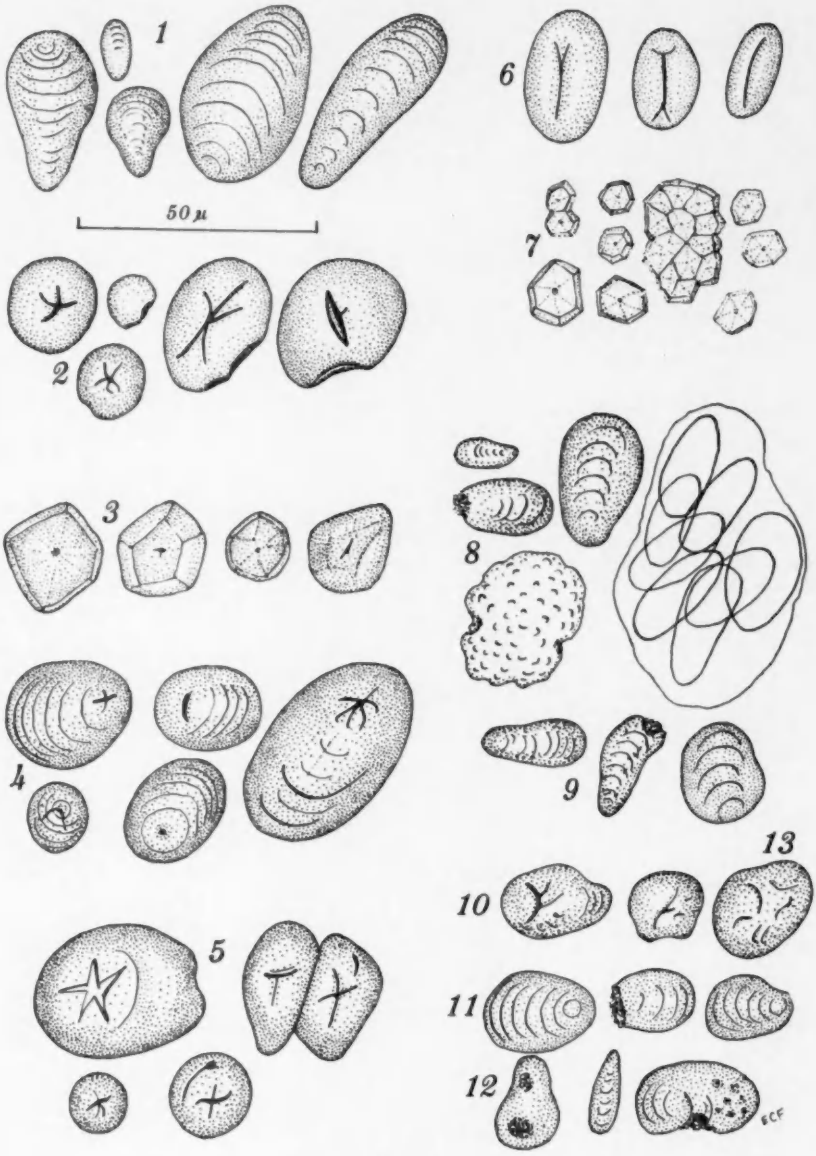

PLATE I

PLATE II. Habit sketches, all drawn to same scale, of *Entamoeba histolytica* trophozoites with ingested starch grains: *1*, plantain; *2*, yucca; *3*, maize; *4*, Irish potato; *5*, white sweet potato; *6*, wheat; *7*, rice. In each study *a* represents an early subculture; *b*, a later subculture of the ameba with the particular starch substrate.

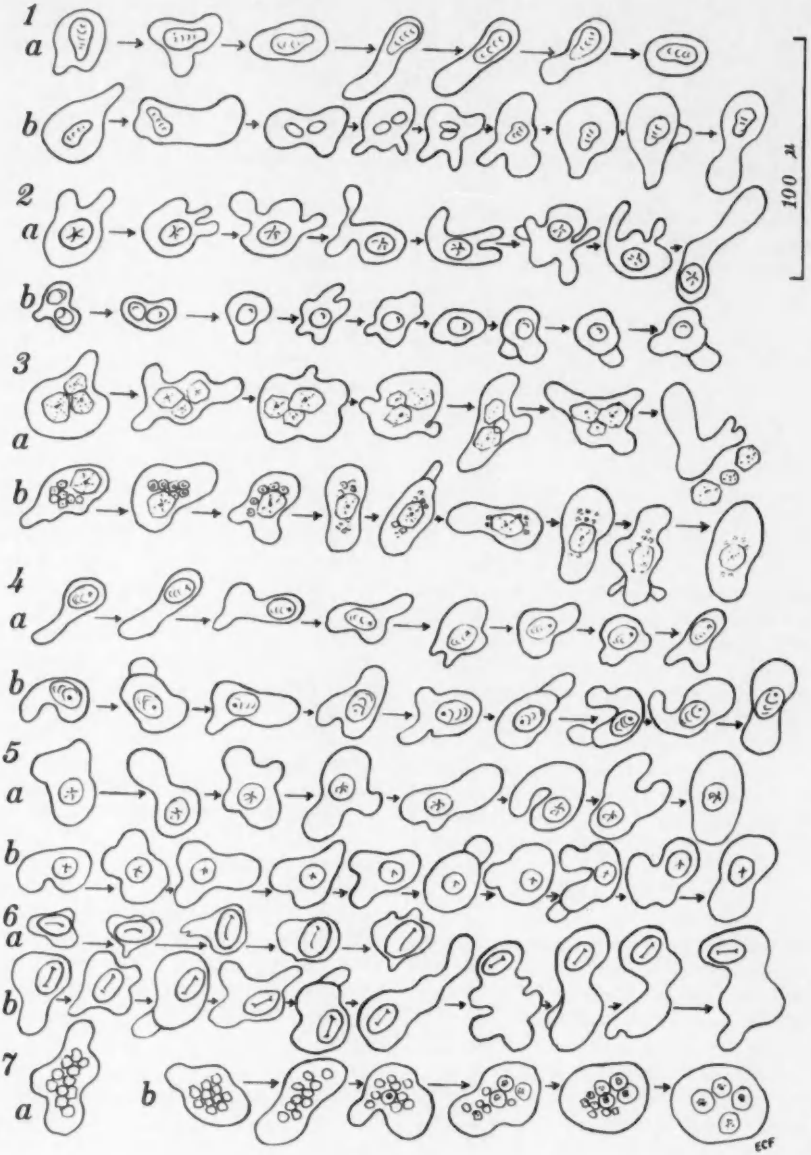

PLATE II

THE POLYANTHELMINTIC ACTION OF DITHIAZANINE IODIDE

Verification of its Effectiveness in the Most Common Helminthiases in Guatemala

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A few years ago we reported[1] on a survey of more than 250,000 stool examinations carried out over a 10-year period in the Parasitology Laboratory of the "Rodolfo Robles" Institute of Tropical Diseases. It was found that the approximate parasitological index of worm infection in the city of Guatemala ranged from 40 to 60%, about 80% in the rural areas, sometimes reaching 100% in heavily infected rural areas. From these data we can assume that more than 66% of the population of the country are infected with intestinal helminths, the most common being *Ascaris* and *Trichuris*. Recently[4] we have verified that *Necator americanus*, as well as *Ancylostoma duodenale*, are commonly found and coexist with other intestinal helminths.

We have been interested in the chemotherapeutic advances in the treatment of intestinal helminthiases as well as in the use of preventive measures that tend to lessen or possibly eradicate helminthiases. Our earlier experiences with anthelmintic drugs, such as glycerinated hexylresorcinol enemas in the treatment of trichocephaliasis and the use of diethylendiamine derivatives in the treatment of ascariasis have been reported.[1, 2, 11, 12]

In 1956 we had the opportunity to cooperate in clinical trial studies conducted in Guatemala with a new anthelmintic, Compound 01748,* in 55 cases of ascariasis, trichuriasis, and *Necator americanus* infection. In the clinical study done by Cordón[5] Compound 01748 was found to be effective against *Ascaris* and *Trichuris* but did not prove to be effective in cases of necatoriasis.

The anthelmintic activity of Compound 01748 (dithiazanine iodide) has been demonstrated by McCowen *et al.*,[7] against whipworm, *Ascaris*, hookworm, and pinworm infections in dogs, cats, and mice. Dogs have tolerated doses of 50 to 200 mg per kg of weight, apparently without any harm. In studies of sustained administration, dogs have tolerated doses of 9 to 13 mg per kg for 5 months, without any harmful effects. Frye *et al.*,[6] obtained high rates of cure with this new trichuricide in unquantitated, subclinical, *Trichuris trichiura* infections. Concurrently, the anthelmintic spectrum of dithiazanine iodide has been explored. A cure rate of 89% was reported by Swartzwelder *et al.*,[9] in a series of cases of human strongyloidiasis treated with this drug. The rigid criteria for cure were the absence of diagnostic forms in a series of examinations of both stools and duodenal fluids after therapy. Miller *et al.*,[8] employed dithiazanine iodide successfully for the treatment of enterobiasis. Evidence of the effective broad spectrum or polyvermicidal activity of dithiazanine iodide against common intestinal helminths was reported by Swartzwelder *et al.*[10]

Based on the encouraging clinical reports by the investigators at Louisiana State University School of Medicine, a new study was set up to amplify our observations of 1956 with new material (CAP coated tablets dithiazanine iodide) supplied by the Lilly Research Laboratories of Indianapolis, Indiana.

MATERIAL AND METHODS

From January to June, 1958, 100 patients with helminthiases were treated in the Outpatient Dispensary of the Parasitology Division of the "Rodolfo Robles" Institute of Tropical Medicine, the Tropical Diseases Outpatient Clinic of the General Hospital, and certain patients from private practice (FJA). The patients were of both sexes and their ages ranged from 2 years and 4 months to 53 years. No special preparation for treatment was given the patients, nor any special diets. With the exception of *Enterobius*, in all cases quantitative stool examinations were made using the Stoll technic before and after treatment. Most of the patients were treated with exploratory dosages in the beginning of our series. Adults were administered dithiazanine iodide in doses averaging 200 mg

* Dithiazanine iodide in gelatin capsules of 100 mg supplied by Dr. J. G. Evans, Medical Research Co-operation Division, Lilly Research Laboratories, Indianapolis, Indiana.

two or three times daily for 5 days, or for longer periods in some cases. In children the exploratory dosage was administered in accordance with their weight.

RESULTS

In 100 patients treated, 59 had infections due to a single parasite, and 41 had multiple infections, due to two, three, or four helminths. The total number of infections was 154; 124 infections were due to nematodes and 30 due to cestodes.

The cure rate with dithiazanine iodide was 100% in enterobiasis and strongyloidiasis; 86.79% in ascariasis; 85.72% in trichuriasis; and 61.53% in uncinariasis. The egg reduction after treatment amounted to 97.99% for *Ascaris*, 93.13% for *Trichuris*, and 88.16% for *Uncinaria*. In the cestode infections, the cure rate for *Hymenolepis nana* was 80%, the egg reduction being 88.89%. Patients with *Taenia saginata* and *Hymenolepis diminuta* were not considered cured since the presence of scolices could not be confirmed in the feces by means of a sieve after treatment.

We are aware that the incidence of nausea and vomiting with the use of dithiazanine iodide has varied considerably among investigators from different areas; however, in our experience only 9 out of 100 patients treated (9%) had nausea and vomiting, and these side-effects were not severe enough to require interruption or discontinuation of treatment.

SUMMARY AND CONCLUSIONS

The polyanthelmintic efficacy of dithiazanine iodide was proved in the treatment of nematode infections which are most prevalent in Guatemala: *Ascaris*, *Trichuris*, *Uncinaria*, and *Strongyloides*.

Dithiazanine iodide was found to be effective in 80% of the cases infected with *Hymenolepis nana*. Among cestodes *H. nana* has the greatest incidence in Guatemala. The therapeutic results obtained in cases of *Taenia saginata* and *Hymenolepis diminuta* were not satisfactory.

Cooperation from the patients was excellent; the cases rejected in this evaluation because of failure to complete the course of treatment was less than 5%.

The polyvermicidal action of dithiazanine iodide permits an effective treatment of single or multiple infections in ambulatory patients attending public health dispensaries and in those treated in private practice.

Emphasis should be placed on the fact that for an adequate anthelmintic treatment, it is necessary to carry out quantitative examinations of feces. We prefer the Stoll technic because of its simplicity and accuracy.

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A COMPARATIVE STUDY OF INTRADERMAL TESTS AND STOOL EXAMINATION IN EPIDEMIOLOGICAL SURVEYS ON SCHISTOSOMIASIS MANSONI

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Schistosomiasis is a parasitic disease of wide geographical distribution affecting more than 100,000,000 people in endemic areas. Although this rough estimate is probably true, it is well to remember that when epidemiological surveys are to give a real picture of the prevalence of schistosomiasis in a given region, some difficulties arise owing to the complex biology of the parasite.

The detection of schistosome eggs in the urine (*S. haematobium*) or feces (*S. mansoni* and *S. japonicum*) has been generally used for diagnostic purposes. However, in endemic areas, when the prevalence of the disease is evaluated by stool or urine examination, lower figures than expected are obtained, since most patients are chronic ones and, at this stage, elimination of the eggs becomes increasingly difficult. To overcome such a disadvantage, the intradermal test is now being tried for epidemiological surveys.[1, 3, 4, 14] This method is supposed to be highly sensitive and has many such advantages as saving of time, equipment and personnel. However, comparative studies between stool or urine examination and the intradermal test performed under different epidemiological conditions are lacking and a more accurate standardization of this test is desired.

The results reported in this paper were obtained in a comparison between stool examination and intradermal test, using cercarial and adult worm antigens in an endemic area of schistosomiasis mansoni.

MATERIAL AND METHODS

Epidemiological surveys on schistosomiasis mansoni using stool examination and intradermal tests were performed in the two following groups of individuals living in Belo Horizonte (State of Minas Gerais, Brasil):

Group A. 454 unselected schoolchildren aged from 10 to 15 years, living in area of high endemicity, engaged in farm work and with frequent contact with *S. mansoni* cercariae shed by *Australorbis glabratus* occurring in the irrigation channels.

Group B. 558 unselected military personnel aged from 20 to 46 years, most of them with only occasional contact with water harboring infected snails.

Intradermal tests were performed on the volar surface of the forearm by injecting 0.05 ml of the antigen (cercarial and adult worm extracts) and reading after 15 minutes, following the method of Pellegrino and Macedo[9] in which the outline of the wheal is drawn in ink and transferred by pressure to slightly absorbent paper and then the wheal area is determined. Wheals with areas of 1.2 sq. cm or more were considered as positive reactions, those with 0.9 sq. cm or less as negative, and the reactions with 1.0 or 1.1 sq. cm wheals were recorded as doubtful.

The antigens were prepared with desiccated *S. mansoni* cercariae[11] or adult worms,[12] as previously described and used in a 1:1000 concentration.

Stool examinations were carried out according to the technique of Hoffman, Pons and Janer.[2] At least 2 hours were allowed for sedimentation. A single slide with 0.1 ml of sediment for each sample of group A (children), and two slides for group B (adults), were microscopically examined under low-power magnification.

RESULTS

Group A (children). Table 1 shows the results obtained with a single stool examination and the intradermal test using adult worm and cercarial antigens. The data show that a single stool examination was positive for *S. mansoni* eggs in 298 children (65.64%) whereas skin tests were positive in 262 (57.71%) and 250 (55.07%) with cercarial and adult worm antigen, respectively. In 289 children (63.66%) the cutaneous test was positive either with one or both antigens. In the group of 298 children with the first stool examination positive for *S. mansoni* eggs, the positivity of the intradermal test was surprisingly low: 67.11% for cercarial antigen and 65.10% for adult worm antigen.

In 62 children with positive cutaneous test and negative stool examination, the latter

TABLE 1

Group A (children)

Intradermal test*	Stool examination		Totals	
	Pos.	Neg.	No.	%
Pos.				
C.....	200	62	262	57.71
A.W.....	194	56	250	55.07
Neg.				
C.....	51	81	132	29.07
A.W.....	52	82	134	29.51
Doubtful				
C.....	47	13	60	13.22
A.W.....	52	18	70	15.42
Totals..	298 (65.64%)	156 (34.36%)	454	

* C. = cercarial antigen; A.W. = adult worm antigen.

diagnostic method was repeated at different times and in 52 of them *S. mansoni* eggs were found.

When the skin reactions were classified as negative, doubtful and positive, a comparative study as between cercarial and adult worm antigen revealed agreement of the results in 351 cases (77.32%). There were no statistically significant differences between the percentages of positive, doubtful or negative readings obtained with the two antigens. There was also no statistically significant difference between the mean areas of the wheals obtained with cercarial and adult worm antigens. In 350 children with positive stool examination the mean areas and standard errors were: for cercarial antigen, 1.38 ± 0.03 sq. cm; for adult worm antigen, 1.35 ± 0.02 sq. cm. The correlation coefficient for the areas observed with the two antigens was r = +0.646, statistically significant at the 0.1% probability level.

Group B (adults). Table 2 shows that in 135 cases (24.19%) stool examination revealed *S mansoni* eggs, whereas the intradermal test was positive in 295 (52.87%) and 287 (51.43%) using cercarial or adult worm antigen, respectively. In 299 cases the cutaneous test was positive with at least one antigen.

In 174 military personnel with positive skin test and negative stool examination, it was possible to repeat feces examination several times. *S. mansoni* eggs were found in 112 cases (Table 3). In addition to these 112 cases, in two cases rectal biopsy was positive, eight had had positive stool examination in the past and three had submitted previously to specific treatment. Twenty two of 45 cases with positive intradermal test and repeated negative stool examination gave positive complement-fixation test with adult worm antigen (Pellegrino and Freitas, 1958, unpublished). Therefore, parasitological or serological evidence of schistosome infection was obtained in 147 of 174 individuals with positive skin test and negative first stool examination.

A comparison of cercarial and adult worm

TABLE 2

Group B (adults)

Intradermal test*	Stool examination		Totals	
	Pos.	Neg.	No.	%
Pos.				
C.....	116	179	295	52.87
A.W.....	116	171	287	51.43
Neg.				
C.....	12	223	235	42.11
A.W.....	12	228	240	43.01
Doubtful				
C.....	7	21	28	5.02
A.W.....	7	24	31	5.55
Totals..	135 (24.19%)	423 (75.81%)	558	

* C. = cercarial antigen; A.W. = adult worm antigen.

TABLE 3

Repeated stool examination in adults with positive skin test and negative first stool examination

Repeated stool examination	Positive
2nd	42
3rd	25
4th	10
5th	13
6th	7
7th	4
8th	8
9th	2
10th to 15th	1
Total pos.....	112

antigens showed agreement of the results in 523 cases (94.35%). The differences between the percentages of positive, negative, and doubtful results obtained with the two antigens were not statistically significant. In 260 patients with parasitological evidence of *S. mansoni* infection there was no statistically significant difference between the mean areas of the wheals (1.97 ± 0.05 sq. cm for cercarial antigen and 1.91 ± 0.04 for adult worm antigen). The correlation coefficient (r = +0.753) for the areas observed with the two antigens was statistically significant at the 0.1% probability level.

DISCUSSION

Analysis of the results showed marked differences in behavior of the two groups. Whereas in group A (children) the positivity rate obtained with a single stool examination (65.64%) was slightly higher than that observed with cercarial (57.61%) or adult worm antigen (55.07%), in group B (adults) skin tests gave much better results than stool examinations, even if it is considered that two slides for each sample were examined. Actually, of 558 adults, only 135 (24.19%) showed *S. mansoni* eggs after a single stool examination, whereas the skin test was positive in 295 (52.87%) and 287 (51.43%), using cercarial or adult worm antigen, respectively. This aspect has already been referred to by other workers.[3, 5, 7, 8, 15]

The results presented above make it clear that in any epidemiological survey using stool examination and/or intradermal test it is of primary importance to consider the age distribution in the group. In addition to age, other factors related to specific local conditions certainly play a role on the results obtained with both diagnostic methods.

Several techniques for stool examination proved to give good results in the diagnosis of Manson's schistosomiasis. A great deal of experience collected in epidemiological surveys, chiefly in Brazil,[13] shows that the method of Hoffman, Pons and Janer,[2] by its simplicity and low cost, can be efficiently used for mass examination. The general employment of this method would, therefore, be of utmost importance for the standardization of stool examination and proper comparative studies of parasitological and immunological methods of diagnosis.

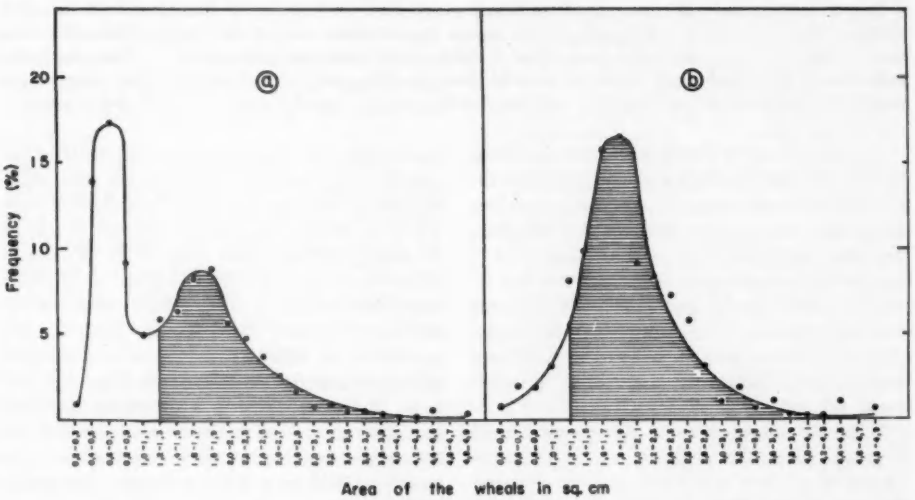

FIG. 1. *a*. Frequency distribution of the wheal areas obtained in group B (adults) with cercarial antigen. The crosshatched area represents the proportion of individuals with positive skin test. The overlapping of the two frequency distribution curves, where the left one represents the individuals with negative and doubtful skin tests, is clearly shown. *b*. Frequency distribution of the wheal areas in 260 adults of group B, with parasitological evidence of *S. mansoni* infection. Observe the small area representing negative reactors. Similar results were obtained with adult worm antigen.

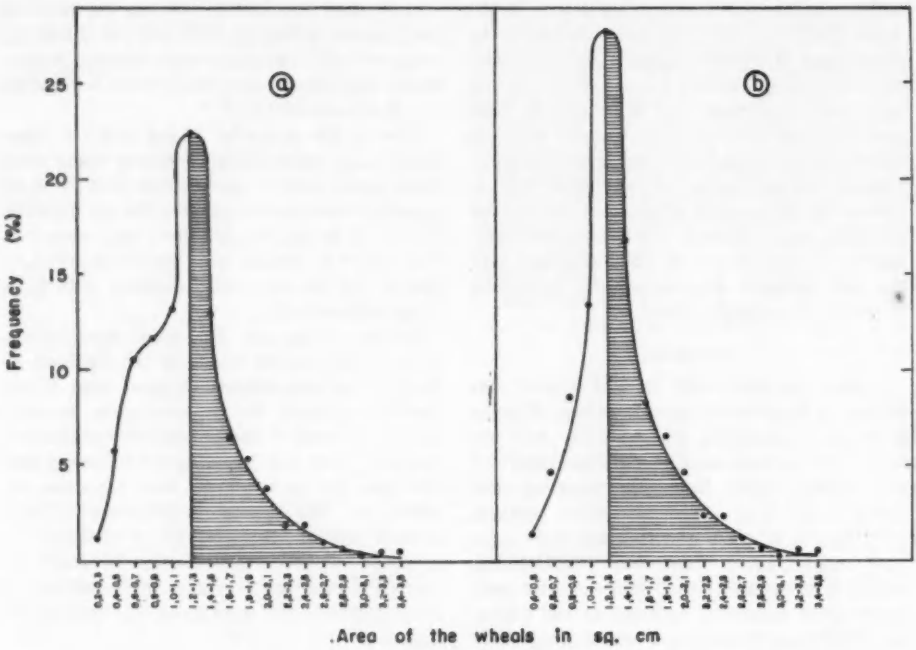

FIG. 2. *a*. Frequency distribution of the wheal areas obtained in group A (children) with cercarial antigen. There is a wide overlapping of the curves representing positive and negative reactors. The crosshatched area represents the proportion of children with positive skin reaction. *b*. Frequency distribution of the wheal areas in 350 children of group A with positive stool examination. Observe the relatively great area of negative skin reactors. Similar results were obtained with adult worm antigen.

As pointed out by Pellegrino,[6] there is a great need for a more accurate standardization of the intradermal test. Actually, a critical review shows that each author prepares and standardizes his own particular antigen and that a number of criteria have been used for the interpretation of results. Recently, a new approach to this problem has been suggested: determination of the wheal area 15 minutes after 0.05-ml intradermal injection of a cercarial or adult worm extract previously standardized.[9, 10]

When the data obtained in surveys are plotted in graphs where the ordinates represent the frequency of a given wheal area and the abscissae the size of the areas, a clear picture of the prevalence of the disease can be obtained. Two different and slightly overlapping frequency curves were obtained in group B (adults), as shown in Figure 1, *a*. The left-hand curve shows the proportion of individuals with negative reactions, and the right-hand one the proportion of positive reactors. In group A (children) there is a wide overlapping and the two curves cannot be clearly distinguished (Fig. 2, *a*). This configuration is explained by the fact that children react less intensely than adults when intradermally injected with specific antigen, the proportion of negative reactors among infected patients being greater in children (Figs. 1, *b* and 2, *b*). Obviously, these findings cannot be taken as a general rule and it may be assumed that the curves representing negative and positive skin reactors would vary with each particular group under study.

The intradermal test with cercarial and adult worm antigens, performed in 100 adults and 100 children, living in a nonendemic area gave the following results: *cercarial-antigen*, adults (92

negative, 5 doubtful, 3 positive); children (96 negative, 4 doubtful); *adult-worm antigen*, adults (90 negative, 7 doubtful, 3 positive); children (98 negative, 2 doubtful).

Further studies in different epidemiological circumstances, using stool examination and skin test under standardized conditions, are highly desirable in order to evaluate the real efficiency of those methods in epidemiological surveys.

SUMMARY

The relative efficiency of stool examination (Hoffman, Pons and Janer technique) and intradermal tests with cercarial and adult worm antigens for diagnosing *Schistosoma mansoni* infection was studied in two groups living in an endemic area: a) 454 unselected schoolchildren aged 10 to 15 years and frequently exposed to infection; b) 558 unselected military personnel with only occasional exposures.

In the first group (children), stool examination gave better results than intradermal test. The positivity rate obtained with a single stool examination (65.64%) was slightly higher than that obtained with cutaneous test using cercarial (57.61%) or adult worm antigen (55.07%). In adults, only 24.19% showed *S. mansoni* eggs after a single stool examination whereas the skin test was positive in 52.87% and 51.43% using cercarial and adult worm antigens, respectively.

Among 289 skin-positive children, 276 (95.50%) were positive for *S. mansoni* eggs after one or more stool examinations. In adults, of 174 individuals who reacted positively to the skin test but had been negative at the first stool examination, 147 (84.48%) showed parasitological or serological (complement-fixation test) evidence of *S. mansoni* infection.

Cercarial or adult worm antigens gave similar results in both groups studied.

The need for further studies in different epidemiological circumstances, using skin test and stool examination under standardized conditions in order to evaluate the real efficiency of these methods in epidemiological surveys, is emphasized.

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EPIDEMIOLOGICAL STUDIES ON HUMAN TRICHOMONIASIS

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Human trichomoniasis is caused by *Trichomonas vaginalis* which is found in the urogenital tract of both men and women.[3, 15] Men harbouring this parasite may have an acute or chronic urethritis or prostatitis but ordinarily have no symptoms at all and are completely unaware of the infection.[15] Women may also harbour the parasite without any symptoms but they usually have an increased vaginal discharge and itching or burning of the genitalia. This may vary from an occasional mild episode just before or after menses to a severe, incapacitating vulvo-vaginitis.[3, 5]

While a great deal has been written on this infection, many aspects of the disease have not been adequately explained. The epidemiological studies reported here were undertaken in conjunction with other investigations to ascertain the prevalence of *T. vaginalis* in different groups of women, the association with other parasitic infections and clinical symptoms as well as methods of transmission.

MATERIALS AND METHODS

Studies were made on 469 women attending a gynecology clinic at the Columbia Hospital for Women in Washington, D. C., and on 1,848 female employees of the National Institutes of Health who participated in one or more of three annual vaginal-cytology surveys conducted by the National Cancer Institute.[6] More detailed studies were made on 97 women found to have trichomoniasis in that survey or who were referred to the *Trichomonas* project because of vaginal discharge or itching.[5] Husbands and daughters of women infected with *T. vaginalis* were examined and interviewed whenever possible.

Columbia Hospital for Women. One to four afternoon gynecology clinics per month were visited at the Columbia Hospital for Women in Washington, D. C., between 1954 and 1957. One member of the *Trichomonas* project staff obtained all of the cultures while the hospital physicians were making the pelvic examinations. Patients usually were unselected to the extent that an effort was made to get cultures on all patients examined. This was not possible in all cases as several physicians were working simultaneously. The sample, therefore, may be slightly biased in favor of patients with vaginal complaints since in these cases the examining physicians would often wait until cultures were made, before passing to other patients.

Vaginal cultures were obtained with cotton-tipped applicators inserted through a vaginal speculum. These applicators were then placed into tubes of NIH trypticase-liver-serum medium,[6] left at room temperature until the clinic closed, when they were transported to the National Institutes of Health and incubated at 37°C. Tooth-pick scrapings from between the teeth were placed in Locke-egg medium for cultures of the oral trichomonad, *T. tenax*.

One member of the *Trichomonas* project staff interviewed the patient regarding vaginal symptoms and hygiene. Later, the hospital record was reviewed and all of the data entered on marginal punch cards for analysis.

Women employed at the National Institutes of Health. Three surveys for vaginal trichomoniasis were conducted in collaboration with the National Cancer Institute's annual cancer detection surveys on women employed at NIH. The women were instructed not to douche or use vaginal medication for 24 to 48 hours before examination. The vaginal secretion for the cytology smear was obtained from the vagina by means of a curved, glass pipette with a rubber bulb.[9] After preparation of the smear the pipette was used to inoculate a culture by rinsing it in a tube of NIH TLS medium.[6] Approximately once an hour the accumulated cultures were taken to the protozoology laboratory and placed in the 37° C incubator.

In 1955 and 1957 the questions asked the women participating in the surveys were limited to race, age, religion, last menstrual period, obstetric and gynecologic history. In the 1956 survey a few questions were included on vaginal discharge and itching of the genitals and frequency of douching. The data available from

* Laboratory of Tropical Diseases.

these questionnaires, the cytology reports, and the results of the cultures for *T. vaginalis*, vaginal fungi, and *T. tenax* when done, were entered on marginal punch cards.

Analysis of data. In order to simplify statistical analysis, only the first culture made on any woman was considered unless stated otherwise.

The observed frequency of *T. vaginalis* in the various categories was analyzed by means of a special chi-square test correcting for the influence of the various classifications other than the one under analysis to determine the probability of finding the observed frequencies of *T. vaginalis* by chance (Mantel and Haenszel, to be published).

It is realized that this may not be truly representative of the female population of the Washington, D. C., area as a whole since the NIH survey was based entirely on working women and the Columbia Hospital survey was based upon Gyn clinic patients. Furthermore, the NIH survey was limited to the women who voluntarily joined a cancer detection survey.

RESULTS AND DISCUSSION

Prevalence

Race. Other than the observation that vaginal trichomoniasis occurs with much greater frequency in colored than in white women there are few aspects of this infection about which there is universal agreement.[3, 15] *T. vaginalis* was nearly eight times as prevalent in the Negro as in the white women examined at NIH, having been demonstrated in 8.1% of 1,512 white women and in 60.9% of 335 Negro women (Table 1). There was much less difference in prevalence of *T. vaginalis* between the white and colored women examined at the Columbia Hospital Gynecology Clinic. Since there were but 22 white women as compared to 447 colored, no conclusion can be drawn from this. It is suggested, however, that the similar socio-economic status of the white and colored women attending the clinic may be responsible for the similarity in prevalence between the two groups.

Age. The prevalence of vaginal trichomoniasis varied by age of the women to a much greater extent than could be expected by chance (Table 1). Contrary to most other reports[15] the highest prevalences in both races at NIH and in the Negroes at the Columbia Hospital GYN Clinic were found in women in their thirties and forties. No conclusion regarding the age distribution in the white women attending the Gyn clinic is warranted because of their small numbers. The highest percentage of infected

TABLE 1

Prevalence of T. vaginalis *by race, age, and marriage, in women examined at NIH and Columbia Hospital Gyn Clinic, with significance level of χ^2 for each association*

Classification	Survey	No. examined	Women with *T. vaginalis*		Significance level of χ^2
			No.	%	
Race					
White	NIH	1,513	123	8.1	
Negro		335	204	60.9	
White	Clinic	22	7	31.8	
Negro		447	211	47.2	
Age					
White:	NIH				P<.01
younger		408	17	4.2	
30–39		500	53	10.6	
40–49		405	42	10.4	
older		200	11	5.5	
Negro:	NIH				P<.05
younger		87	43	49.4	
30–39		144	97	67.4	
40–49		92	58	63.0	
older		12	6	50.0	
Negro:	Clinic				P<.01
younger		31	8	25.8	
20–29		184	82	44.6	
30–39		150	79	52.7	
40–49		75	42	56.0	
older		7	0	0	
Marital Status					
White:	NIH				ns†
single		387	19	4.9	
married		1,126	104	9.2	
lives with spouse	NIH*	576	47	8.2	ns
separated		141	17	12.1	
Negro:	NIH				ns
single		55	34	61.8	
married		280	170	60.7	
Negro:	Clinic				ns
single		45	23	51.1	
married		402	188	46.8	

* Based on results from 1956 survey only.

† ns = not significant.

women in that group was, however, in those less than 30.

The crude and age-specific rates for the colored employees at NIH were appreciably higher than those for the Negro women attending the Columbia Hospital Gyn clinic. Age-adjusted rates for the two groups were closer together than the crude rates but still were significantly different. The difference between methods of collection of samples was shown not to be a factor by making parallel cultures by both techniques from approximately 50 consecutive patients at the Gyn clinic. The fact that many of the women attending the Gyn clinic douched before coming to the clinic was not considered to be an important factor since the prevalence was actually higher in the women who had douched, and the experience with the volunteer study patients at NIH indicated that douching even with purportedly trichomonicidal preparations did not interfere with the cultural demonstration of the parasites. The transportation of the specimens from the clinic to NIH via car may have agitated the cultures to such an extent that some did not survive but it is felt that the socio-economic status of the two groups may have been the most important factor. Most of the women attending the Gyn clinic were housewives and hence may have had a much smaller exposure to risk from contaminated toilet seats and other nonsexual infection than the working women.

Marriage. There was no significant difference in prevalence of *T. vaginalis* between married and unmarried Negro women in either survey. However, married white women at NIH had a greater frequency of the infection than the single women (Table 1). When this was adjusted for differences in age, religion and obstetrical history, the difference was not statistically significant. It was noted that many of the married volunteer-study patients were no longer living with their husbands and, since in some the irritation caused by the trichomoniasis had apparently resulted in marital discord with ensuing separation, an analysis was made to see if such an effect could be supported statistically. A simple chi-square test indicated the difference to be significant but when adjustments were made for age and other factors, the difference was not statistically significant. There was no distinction made in this analysis, how-

TABLE 2

Prevalence of T. vaginalis *by history of pregnancy, status of pregnancy, religion and abnormal vaginal cytology in women examined at NIH and Columbia Hospital Gyn Clinic with significance level of χ^2 for each association*

Classification	Survey	No. examined	Women with *T. vaginalis*		Significance level of χ^2
			No.	%	
Pregnancy					
History of pregnancy					
White:	NIH				ns†
no		327	24	7.3	
yes		799	80	10.0	
Negro:	NIH				ns
no		106	66	62.3	
yes		229	138	60.3	
Negro:	Clinic				ns
no		117	58	49.6	
yes		330	153	46.4	
Type of pregnancy					
White:	NIH*				ns
term only		377	34	9.0	
abortions		142	15	10.6	
Status of pregnancy					
Negro:	Clinic				P<.05
first trimester		64	27	42.2	
post partum		29	6	20.7	
Religion					
White:	NIH				P<.01
Protestant		1,059	101	9.5	
Catholic		350	15	4.3	
Jewish		81	5	6.2	
other or not stated		23	2	8.7	
Negro:	NIH				ns
Protestant		279	174	62.4	
Catholic or not designated		56	30	53.6	
Abnormal vaginal cytology					
Both races	NIH				
class II or greater		40	14	35.0	P<.01
class 0 or I		1,808	313	17.3	

* Based on results from 1956 survey only.

† ns = not significant.

ever, as to whether the separation was due to divorce or death.

Pregnancies. The infection was slightly more common in Negro women of both surveys who had never been pregnant than in those who had (Table 2). This was not true of the white women except for ages 30 through 49. In no instance, however, was the difference significant.

There was no difference between prevalences of *T. vaginalis* in women with a history of an abortion or miscarriage and in those who had had only term deliveries. While based on only a relatively few women, it is of interest that the Negro women attending the Columbia Hospital Gyn clinic for postpartum examinations had a significantly lower prevalence of vaginal trichomoniasis than did those who were in the first trimester of pregnancy (Table 2). It is, of course, impossible to ascertain from these data whether the trichomonads disappeared during the course of the pregnancy or during the sudden endocrinological changes occurring after delivery.

Religion. One of the items covered by the National Cancer Institute questionnaire which was filled out for all women participating in the cancer-detection survey concerned the individual's religion. This presumably was included to get statistical data to evaluate the assertions that cancer of the cervix occurs less frequently in Jewish women. There were too few Jewish women in the NIH survey to evaluate the prevalence of *T. vaginalis*. It was observed, however, that they did have a lower frequency than did the white Protestant women. It was further ascertained that the white Catholic women had an even lower frequency (Table 2). This was especially marked in the unmarried Catholic women who had one-eighth the frequency of unmarried Protestants. The difference between the religious groups was statistically significant with $P < 0.01$ even when adjusted for age differences, marital status, and obstetrical history. No such association was found in the Negro women.

Association with Clinical Manifestations

Ninety-five of the 447 colored women examined at the Gyn clinic had vaginal discharge listed as one of their chief complaints in their clinical records. While 53.7% of such women had *T. vaginalis* as compared to 45.5% of those who did not complain of discharge, this difference was not statistically significant. Furthermore, 61 women who were listed as having a vaginitis by the clinic physician did not have a significantly greater frequency of vaginal trichomoniasis than did those without vaginal inflammation. There was, therefore, no demonstrable association of *T. vaginalis* with complaint of vaginal discharge or presence of vaginitis in the Negro women attending the Gyn clinic. This does not indicate that this infection does not cause these conditions but rather that it is not the only cause.

TABLE 3

Prevalence of T. vaginalis *and vaginal fungi in white female employees at NIH in the 1956 cancer-detection survey*

Classification	No. examined	*T. vaginalis*		Significance of χ^2	Vaginal fungi		Significance
		No.	%		No.	%	
History of discharge:				.001			ns*
yes	200	46	23.0		49	24.5	
no	708	29	4.1		149	21.0	
Discharge:				.05			ns
past year	138	38	27.5		33	23.9	
prior only	62	8	12.9		16	25.8	
History of itching:				.001			ns
yes	142	32	25.5		35	24.6	
no	766	43	5.6		163	21.3	
Itching:				.05			ns
past year	73	21	28.8		19	26.6	
prior only	69	11	15.9		16	23.2	

* ns = not significant.

The white women who gave a history of vaginal discharge in the 1956 survey at NIH had nearly six times the frequency of *T. vaginalis* found in those who denied a discharge (Table 3). The white women with such a history who stated that they had been free of vaginal discharge during the previous year had less than half the frequency of infection found in those who had such symptoms during the preceding 12 months.

There was no difference in prevalence of vaginal fungi (unidentified) between white women with or without a history of vaginal discharge and those who had had a discharge only before, or during, the past year. It is therefore, assumed that vaginal infection with *T. vaginalis* is an important cause of discharge in the white women at NIH but that infection with vaginal fungi is not.

The same relations also were observed in regard to history of genital itching in the white women of the 1956 survey (Table 3). Similar analysis of association of *T. vaginalis* with history of discharge or itching in Negro women at NIH showed a similar trend but the differences were no greater than could occur by chance.

Association with Other Parasites

T. hominis. In the past it was thought by many investigators that the human vaginal, intestinal and oral trichomonads were all the same species and that the genital infection came from the rectum. Experimental implantation into the vagina of volunteer women has indicated that the 3 trichomonads are distinct species.[15] This view is supported by studies of Bland and Rakoff[4] and others who have failed to show an appreciably higher percentage of intestinal or buccal trichomonads in persons with *T. vaginalis* than in the population at large.

Two of 67 women with vaginal trichomoniasis at NIH were demonstrated to be infected with *T. hominis* by culture of stool specimens in Locke-egg medium. Both of these women were among the 37 white women in the study series. While this is a greater prevalence than is found in routine examinations, the numbers are too small to be considered significant.

T. tenax. Tooth-pick scrapings from between the teeth of 176 white and 406 Negro women were cultured for *T. tenax*. The oral trichomonad was cultured from 72 (33.8%) of 213 women with *T. vaginalis* and 71 (19.2%) of 369 women without vaginal trichomoniasis. The association of the two species of *Trichomonas* was far greater than could have occurred by chance ($P < .001$) which may indicate that they are actually the same species or that one may survive in the other's habitat.

Vaginal fungi. All vaginal cultures were examined for *Candida albicans* and similar fungi in addition to trichomonads. Vaginal fungi were found only about two-thirds as frequently among Negro women as *T. vaginalis*, but were nearly three times as common as the trichomonads in the white women at NIH.

Vaginal fungi which were not classified were cultured from nine (12.0%) of 75 white women with *T. vaginalis* and from 187 (22.4%) of 833 white women without vaginal trichomoniasis at NIH. The prevalence of vaginal fungus was significantly lower in white women ($P < .05$) examined at NIH who were infected with *T. vaginalis* but no such difference was observed in the Negro women examined either at NIH or the Gyn clinic.

Eighty-five vaginal cultures from the 1957 survey containing what our laboratory reported as vaginal fungus (unclassified) were sent to the Clinical Pathology Laboratory for identification. Twenty-four were identified as *C. albicans*, ten as other species of *Candida*, eight as *Rototorula*, one as *Ustilago*. No fungi were found in two, and 41 were reported to be "yeast—probably not *Candida*, unable to identify by routine laboratory procedure." Thus, only about 25% of those reported to have vaginal fungi in the above surveys had *C. albicans*, which is the species generally considered pathogenic.[3] It is possible, therefore, that a correlation between fungus infection and vaginal discharge might have been found if the analysis reported previously had been limited to *C. albicans*.

Association with Abnormal Vaginal Cytology

T. vaginalis is known to interfere with the interpretation of vaginal smears made for endocrine studies and for detection of cancer.[3, 9, 13] In addition, it has been suggested that infection with this parasite actually may be a factor in the development of cervical carcinoma by keeping up a "chronic irritation which may produce the metaplastic changes in the cervical epithelium that occur in the shift from the normal to the malignant."[8] In this connection Davis[7] reported, on the basis of 7,699 cancer-detection smears, that women with both malignant cells and *T. vaginalis* were on the average 15 to 16 years younger than those with malignant cells alone.

The cytology smears from the 1955 survey were examined by the cytology unit in Memphis, Tennessee while those from the 1956 and 1957 surveys were read by the Washington Cytology Unit of the National Cancer Institute. All smears with cytological atypias, Papanicolaou classification class II and above have been considered atypical for the purposes of this analysis.

White women with vaginal trichomoniasis had four times as many cytological atypias as those without this infection (Table 2). No such differ-

ence was noted among the Negro women examined, except that those between 30 and 39 had a high prevalence of infection with *T. vaginalis* (60.9%). This, however, may have masked such an association. Even when statistical adjustments were made for variations in prevalence due to race, age, marital status, obstetrical history and religion, the observed association between *T. vaginalis* and cytological atypias could be expected to occur by chance less than once in a hundred observations.

A similar association was observed between trichomoniasis and cytological atypias, Papanicolaou class III and IV, as well as with cases of proven malignancy; however, the number of cases observed was smaller and no conclusion concerning them is warranted at this time.

These data tend to support the concept of Davis and Grand[8] and of Ayre[1] that chronic inflammation may contribute to the production of cervical carcinoma, but are also compatible with the concept that some unrelated factor which leads to the production of carcinoma renders the vagina a more favorable habitat for the *T. vaginalis*.

METHODS OF TRANSMISSION

Since the infection occurs in both males and females it has been assumed that transmission may take place during sexual intercourse and studies on sexual partners of infected women have shown that significant numbers of them harbor the parasite.[15]

T. vaginalis was cultured from urethral swabs or prostatic secretion from 13 (41.9%) of 31 spouses of infected women who submitted to examination. This is probably biased since men with symptoms would probably be most apt to volunteer for examination.

A further demonstration of the important role of the sexual partner was the greater difficulty of curing women living with their spouse. Three times as many single, divorced or widowed women in our series were cured of their trichomoniasis as women living with their husbands.

Coitus is not, however, the only method of transmission since trichomoniasis is also found in infants and young children.[15] Kleegman[11] reported that 9% of her cases were in virgins. None of six daughters from four infected white women at NIH was found to have vaginal trichomoniasis.

TABLE 4

Results of culture of cloth used to cleanse external genitalia of women infected with T. vaginalis

Hours before cloth cultured	No. of tests	Positive for *T. vaginalis*
0–1	23	14
1–2	40	16
2–3	40	15
3–4	8	1
4–5	8	3
5–6	6	1
6–7	6	0
7–8	5	2
18–20	10	0
21–24	9	1

It is thus apparent that some other mechanism must also operate to spread the disease. Swift[14] demonstrated living trichomonads in drops of vaginal discharge that had been allowed to dry for up to 3 hours on a glass slide, and Kessel and Thompson[10] obtained cultures of *T. vaginalis* from samples of vaginal exudate after drying on enameled blocks of wood for up to 6 hours. Trussel[15] reported that trichomonads had been cultured from toilet seats after several infected women had used them. It would thus seem logical to assume that women can become infected by sitting upon a contaminated toilet seat. McCullagh,[12] in England, estimated that 80% of the cases are acquired from toilet seats.

To test the hypothesis that communal use of other fomites, such as wash cloths, might spread the disease the external genitalia of 38 women infected with this parasite were wiped with pieces of wash cloth wet with tap water and then allowed to stand at room temperature in a moist environment for varying periods of time. Positive cultures were obtained from these pieces of wash cloth after standing for 23 hours (Table 4). It is, therefore, reasonable to conclude that *T. vaginalis* is often transmitted by fomites, particularly among individuals with a poor concept of hygiene and sanitation.

SUMMARY

1. The epidemiological studies reported here were undertaken to ascertain the prevalence of *T. vaginalis* in different groups of women, the

association with other parasitic infections and clinical symptoms as well as methods of transmission.

2. *T. vaginalis* was nearly eight times as frequent in Negro women as in white employees who participated in a cancer-detection survey at the National Institutes of Health, but there was no significant difference between Negro and white women attending a Gyn clinic.

3. The prevalence of vaginal trichomoniasis was greatest in women of both races 30 to 49 years of age.

4. No significant alteration in the prevalence of *T. vaginalis* could be attributed to marital status or history of pregnancy except that Negro women attending the Gyn clinic for postpartum examinations had a prevalence of vaginal trichomoniasis which was much lower than could be expected by chance.

5. White Protestant women at NIH had a significantly higher prevalence of vaginal trichomoniasis than the nonProtestant white women ($P < .01$).

6. Women with a history of vaginal discharge or pruritis who had had no symptoms during the preceding year had a much lower prevalence of *T. vaginalis* than did those with a persistant discharge. No such difference was noted regarding the prevalence of vaginal fungi.

7. *T. tenax* and *T. vaginalis* occurred in the same individuals much more frequently than could be attributed to chance. ($P. < .001$)

8. Vaginal smears showing cytological atypias, Papanicolaou class II and above, were much more prevalent in women with vaginal trichomoniasis than in uninfected women. This was especially marked in white women. A similar association was observed between trichomoniasis and cytological atypias, Papanicolaou class III and IV, as well as between trichomoniasis and cases of proven malignancy. However, the number of cases observed was small and no conclusion concerning them is warranted at this time.

9. The importance of the sexual partner in the epidemiology of trichomoniasis was emphasized by culturing *T. vaginalis* from nearly half of the spouses of infected women who submitted to examination, and by the fact that three times as many single, divorced or widowed women in our series were cured of their trichomoniasis as were women living with their husbands.

10. Transmission by means of communal use of fomites, such as wash cloths, was shown to be possible by culturing *T. vaginalis* from pieces of wash cloth after standing at room temperature for up to 23 hours after being used to cleanse the external genitalia of infected women.

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SCHISTOSOMIASIS OF SPLEEN WITH ABSCESS FORMATION AND INFARCTION

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Very few cases of massive involvement of the spleen by schistosome eggs have been reported in the literature. It was therefore considered worthwhile to report this case.

CASE REPORT

Patient B.B.E., a 23-year-old Egyptian male, was admitted to the American Mission Hospital, Tanta, Egypt, on March 28th, 1955. The chief complaint was the presence of a painful and tender mass in the left upper quadrant of the abdomen. The patient's illness had started 2 months previously, with sudden onset of a persistently high fever, together with drowsiness, headache and anorexia. After about 10 days the fever began to drop, following which the patient experienced severe, aching pain in the left hypochondrium, opposite the sternum and in the chest. The pain was referred to both shoulders. Simultaneously the patient noted the presence of a swelling in the left, upper part of his abdomen, which was associated with a sensation of heaviness. He consulted a physician at that time, but in spite of 1 month's treatment there was no improvement, apart from subsidence of the fever. In addition, he lost some weight. There were no other symptoms of note.

The patient had contracted urinary schistosomiasis during his childhood. Three years prior to his present illness he had had an attack of hematemesis, for which he was hospitalized, and from which he had recovered. His family and personal histories were noncontributory.

Physical examination showed a wasted, acutely ill, sallow-complexioned male, with a temperature of 98°F, a pulse rate of 100/min, respirations 30/min, and a blood pressure of 120/80. There was marked pallor of the mucous membranes and nail beds. The tongue was dry and fissured. Moderate engorgement of the neck veins was noted. No enlarged lymph nodes were palpated. The heart was regular in rate and rhythm, and the apex beat was in the fourth intercostal space inside the mid-clavicular line. A diastolic shock was felt, and a soft systolic murmur auscultated in the area of the pulmonary valve. By percussion there was dulness from the sixth, left, intercostal space downwards. A few expiratory rhonchi were heard over the lung bases. A tender, fixed and coarsely irregular mass was felt in the left, upper quadrant of the abdomen, which extended medially and downwards to the level of the umbilicus. Its borders were rounded, and a definite notch could be palpated. Posteriorly it stopped short of the renal angle. The mass did not move with respiration. The liver was felt three finger breadths below the right, costal margin in the mid-clavicular line. It was firm, smooth and nontender. There was no free fluid in the abdomen. Examination of the rest of the systems was negative.

Laboratory investigations revealed the following: 1. Hematology—RBC 3,670,000; hemoglobin 9 g (58%); WBC 6,650; differential count: neutrophil segs. 70, monocytes 0, lymphocytes 24, eosinophils 6, basophils 0; coagulation time 5 min.; bleeding time 4 min. 2. Blood chemistry—blood urea 44 mg; cephalin cholesterol test +++. 3. Urinalysis—normal; no schistosome eggs. 4. Fecal examination—eggs of *Ascaris lumbricoides*, but no schistosome eggs. 5. I.V. pyelogram—good functioning of kidneys at 7 minutes with normal findings. Both ureters slightly dilated. The lower third of the right ureter showed minute filling defects due to ureteritis cystica. The urinary bladder was incompletely filled with dye at 25 minutes. 6. Fluoroscopy of chest—slightly prominent pulmonary conus (cor pulmonale). Left diaphragm high and splinted. Both lungs clear.

On the eighteenth day of hospitalization the patient was given a pint of whole blood. Two days later, under general endotracheal anesthesia, the abdomen was opened through a left, upper, paramedian incision. The spleen was found densely adherent to the diaphragm, anterior abdominal wall and the omentum. On separating the

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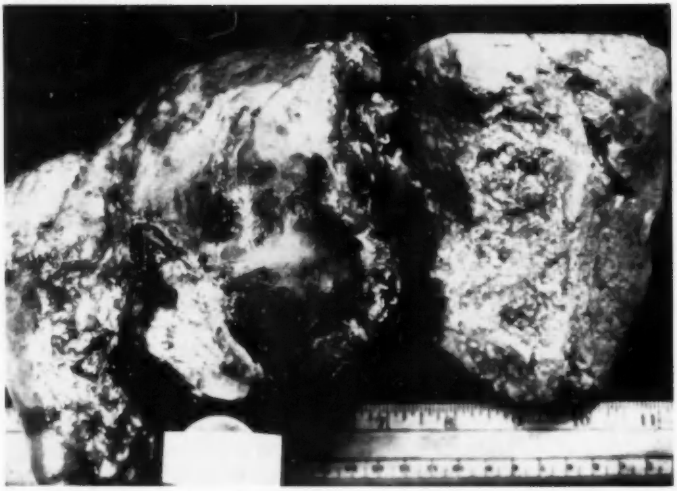

FIG. 1. Gross photograph of spleen. To the right is the necrotic portion found lying free within the abscess cavity at operation.

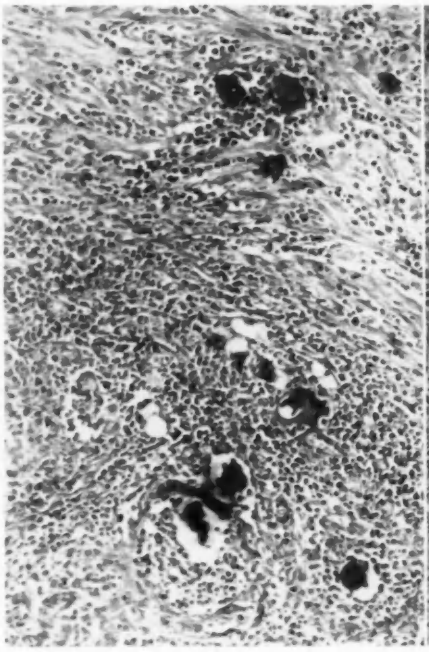

FIG. 2

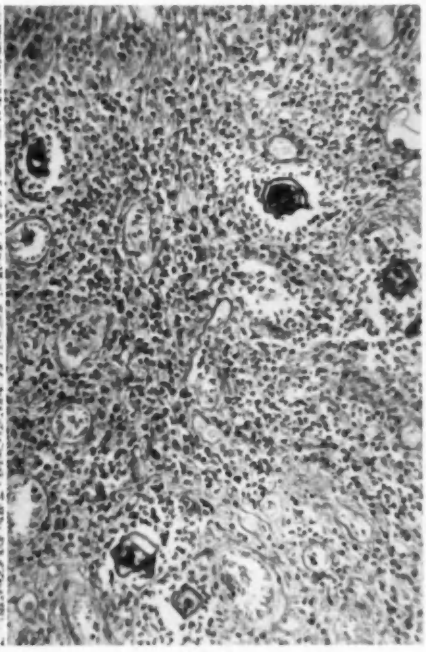

FIG. 3

FIG. 2. Photomicrograph of wall of large abscess in spleen. Toward the top is the fibrous wall, and below is the inner lining of granulation tissue. Scattered throughout are dark-staining schistosome eggs, some of which are phagocytosed by foreign-body giant cells. Hematoxylin and eosin. ×200.

FIG. 3. Photomicrograph of inner lining of granulation tissue of large splenic abscess. Several schistosome eggs are seen. Hematoxylin and eosin. ×200.

spleen from the diaphragm an abscess cavity was opened, from which about 1 L of yellow pus was aspirated. Lying free within this cavity was a large piece of necrotic splenic tissue. After division and ligature of the adhesions, the splenic vessels were tied and the spleen removed. Closure of the abdominal wall in layers, without drainage, was next effected. The patient made an uneventful recovery, and was discharged 8 days postoperatively. He is to date living and in good general conditon.

PATHOLOGY

The spleen weighed 850 g. The entire upper pole, which measured 14 x 8 cm, was a soft, necrotic mass. It had separated from the rest of the spleen, leaving a thickened, yellowish-white abscess membrane attached to the spleen (Fig. 1). Other necrotic areas were present, one close to the lower pole, which measured 5 cm in diameter, and a smaller one at the extreme lower edge of the lower pole, which measured about 3 cm in diameter. The capsule of the spleen was uneven, due to the numerous fibrous adhesions which had been divided during operation. It was greatly thickened, measuring up to 0.5 cm in some areas. The trabecular framework was prominent, and the organ cut with increased fibrous resistance in most areas. The cut surface was reddish-brown, with scattered grayish-white areas of infarction, measuring up to 2 cm or more in diameter. The lymphoid follicles were barely discernible to the naked eye as widely spaced, pinpoint, gray spots. Small hemorrhages were also scattered in the pulp.

Microscopically the capsule of the spleen showed marked fibrous thickening, with moderate infiltration by mononuclear cells. The trabeculae were also greatly thickened and appeared to be more numerous than normal. An advanced degree of pulp fibrosis with overgrowth of the reticulum framework was evident with the tri-

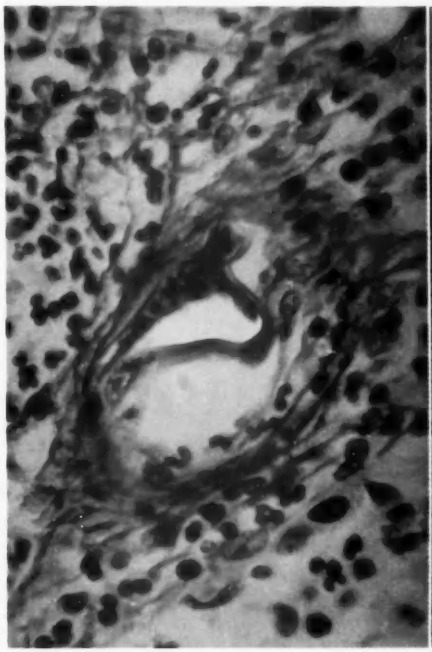

FIG. 4

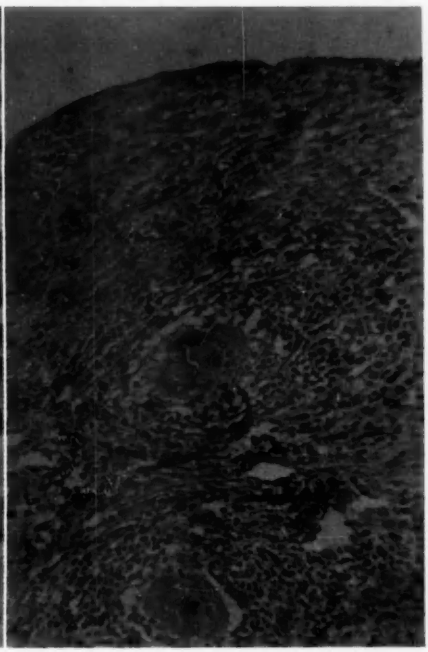

FIG. 5

FIG. 4. Photomicrograph of collapsed nonviable egg within capillary vessel in the granulation tissue of the splenic abscess. Hematoxylin and eosin. ×600.

FIG. 5. Photomicrograph of portion of trabecular artery of spleen, with two schistosome granulomas in its wall. Note chitinous remains of eggs, engulfed by multinucleated giant cells. Hematoxylin and eosin. ×200.

chrome and reticulum stains. The sinusoids were dilated and congested, but contained normal blood elements. Yellowish-brown, refractile, hemosiderin granules were present in abundance, both within the fixed and wandering macrophages, and lying free in the pulp reticulum. Large, recent and old hemorrhages were scattered throughout. Occasional fibro-siderotic nodules (Gamna-Gandy bodies) were also noted. Lymphoid follicles were widely spaced, and generally displayed no germinal centers.

Sections examined from the thick wall of the abscess cavity in the upper pole of the spleen showed it to be composed of bundles of collagen and fibroblasts. Numerous hemosiderin pigment granules were interspersed through the connective tissue, many of them being within macrophage cells. Infiltrating the fibrous connective tissue were numerous chronic inflammatory cells, with predominance of plasma cells (Fig. 2). Lining the inner wall of the abscess cavity was thick, vascular, granulation tissue infiltrated with plasma cells, polymorphonuclear leucocytes, eosinophils, lymphocytes, monocytes, and lipoid and hemosiderin macrophage cells (Fig. 3).

The striking finding, however, was the presence, within this granulation tissue, of numerous *Schistosoma haematobium* and *mansoni* eggs (Figs. 2 and 3). Most of these were nonviable, with distortion, fragmentation or shrinkage of the chitinous shells. Some of the eggs were noted within thin-walled capillaries (Fig. 4). There were occasional foreign-body, multinucleated, giant cells engulfing eggs or their remnants. Schistosome granulomas were sometimes seen. Several smaller abscess cavities, similar to the one described above, were present. Stains for bacteria and fungi were negative.

In one of the sections a large trabecular artery, in the vicinity of the large abscess cavity, showed several schistosome granulomas embedded in its thickened wall (Fig. 5). Isolated eggs not associated with any inflammatory reaction were also seen. In some of the sections, smaller arteries contained recent thrombi, surrounded by varying sized areas of infarction.

DISCUSSION

Eggs have rarely been demonstrated in routine histological sections from the enlarged spleens associated with hepatic schistosomiasis.[1, 6, 7] Several investigators, however, have been able to recover eggs after digestion of splenic tissue with sodium or potassium hydroxide.[4, 5, 7] From the autopsy material at the Kasr El Ainy Hospital, Cairo, Egypt, Sorour,[9] over a 10-year period, never encountered eggs or worms in sections of spleens from bilharzial patients, although he had rarely seen worms in the splenic vein. No investigator has satisfactorily explained the 'immunity' of the spleen to the migration of worms into the splenic vein and its branches.

The only case in the literature in which *S. haematobium* eggs were found in large numbers in the spleen examined postmortem was reported by Blanc and Touzin.[1] To our knowledge the presence of a large number of schistosome eggs associated with abscess formation and infarction of the spleen has not been previously reported.

No worms were noted in the splenic vein or its branches or in multiple sections of the spleen in our case. The presence of schistosome granulomas and eggs in the wall of a large trabecular artery leads us to the conclusion that the eggs reached the spleen as emboli via the splenic artery, after escaping from the pulmonary circulation. That the escape of eggs from the lungs into the systemic circulation is possible has recently been demonstrated by De Faria,[2, 3] who has described the formation of arteriovenous fistulas in pulmonary schistosomiasis. De Faria[3] has also postulated that eggs may pass through normal arteriovenous shunts in the lungs.

Our patient was suffering from both the urinary and intestinal types of schistosomiasis. We believe that he also had chronic pulmonary schistosomiasis, with clinical and radiographic signs of cor pulmonale. While no hepatic biopsy was taken at operation the patient no doubt had hepatic schistosomiasis, with portal hypertension and esophageal varices, from which he had previously had an attack of hematemesis. Apart from the presence of the schistosome eggs and granulomas, the spleen showed advanced fibrocongestive changes, which must have existed long before any eggs reached the spleen. The infarcts in the spleen were associated with thrombi in the smaller branches of the splenic artery, which were probably related to lesions produced by egress of the eggs. We can offer no explanation for the abscess cavities, since no cultures were made from the pus, and since the bacterial tissue stains were negative.

SUMMARY

1. The clinical, laboratory and operative findings in a young Egyptian male suffering from an enlarged, painful and tender spleen are reported and commented upon.

2. Histological examination of the surgically removed spleen showed multiple abscess cavities and infarcts, together with numerous schistosome eggs and granulomas. Both *S. haematobium* and *S. mansoni* eggs were identified in the sections.

3. The schistosome eggs are believed to have reached the spleen as emboli via the splenic artery, after having escaped from the pulmonary into the systemic circulation.

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RESISTANCE OF *SHIGELLA FLEXNERI* TO TETRACYCLINES, CHLORAMPHENICOL AND STREPTOMYCIN

A Study of 131 Freshly Isolated Strains

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Among the enteropathogenic bacteria found in the stools of children over 6 months of age with diarrhea in Mexico City, *Shigella* organisms are the most frequent. In three surveys carried out in the last 15 years in this city, it was found that 91%,[5] 79%,[7] and 76%[6] of the total number of strains of *Shigella* isolated from patients with diarrhea, were members of the subgroup B (*Shigella flexneri*).

Therapeutic problems apparently related with the emergence of resistant strains to the antibiotics which are currently in use have been observed at the Hospital Infantil de Mexico in cases of shigellosis. It has been suggested that the appearance of resistant strains is somehow related with the extensive use of the antibiotics.[2]

It is the purpose of the present communication to report the results of sensitivity tests on various strains of *Shigella flexneri*, isolated in Mexico from the stools of children with diarrhea during the last five years, to five different antibiotics which have been in use at the Hospital Infantil de Mexico during this period of time.

MATERIAL AND METHODS

Strains tested. Two series of cultures of *Shigella flexneri* were tested. The first series included 31 strains isolated during 1953. The second series included 100 strains isolated during the years 1955, 1956 and 1957. The second series included the following serotypes: *S. flexneri* 2a (54 strains), *S. flexneri* 4 (13 strains), *S. flexneri* 6 (12 strains), *S. flexneri* 1b (8 strains), *S. flexneri* 3 (7 strains), and *S. flexneri* 1a (6 strains).

Antibiotics tested. Freshly prepared solutions of the following antibiotics were used: tetracycline, chlortetracycline, oxytetracycline, chloramphenicol and streptomycin.

Test for sensitivity. Each strain of *Shigella flexneri* was tested by the plate-dilution method described elsewhere.[4] Brain-heart-infusion agar, 1.5%, (Difco) was used. The final concentrations of antibiotics were 50, 10, 5, 2.5, and 0.6 μg/ml.

Each agar plate was inoculated with a 3-hour broth culture, using sterile swabs for streaking. This seed culture was prepared by inoculating 10 ml of broth with 0.1 ml of a 20-hour culture.

The end-point was taken as the minimal antibiotic concentration producing inhibition for 20 hours when incubated at 37° C.

RESULTS AND DISCUSSION

Since the over-all results in terms of resistance or susceptibility of *Shigella* organisms to tetracycline, oxytetracycline and chlortetracycline were essentially the same, only the data obtained with tetracycline will be described in this report.

Table 1 shows that none of the 31 strains of *Shigella flexneri* tested in 1953 with tetracycline was resistant to this antibiotic. It is interesting to point out that the use of this substance at the Hospital Infantil de Mexico at the time was limited. During the following years (1955-1957) the use of this antibiotic was extensive and it is of interest to see that concurrently with this wider use of the substance tetracycline-resistant strains appeared with increasing frequency. The emergence of resistant strains of various enteropathogenic bacteria to the action of tetracycline has been described. Huey and Edwards[3] found that among 100 strains of *Salmonella typhimurium* isolated from fowls and 100 similar strains isolated from humans prior to 1948, none was resistant to tetracycline, while among 100 strains isolated from fowls and 100 strains recovered from man after January, 1956, 9% of the former and 5% of the latter were resistant to this antibiotic. Buttiaux *et al.*,[1] studying the tetracycline sensitivity of *Escherichia coli* 0111:B4 strains isolated from hospitalized children, found no tetracycline-resistant strains in 1953, while in 1955, 92.5%

of the strains tested were resistant to the antibiotic.

The significance of the results of the in vitro tests of sensitivity to tetracycline obtained by us may be stressed by the following clinical observations. De la Torre, Olarte, and Joachin[2] treated with tetracycline 13 children with diarrhea and infected with *Shigella* organisms. Nine of the infecting strains were susceptible in vitro to the antibiotic and four were resistant to more than 50 μg/ml. In the former group eight children recovered and in one case no benefit was observed from the antibiotic. In the group with organisms resistant to tetracycline, only one child recovered; the other three cases showed no benefit from the antibiotic. Marberg, Altmann and Eshkol-Bruck[5] also failed in treatment with tetracycline in seven cases of shigellosis in which the strains were resistant to the substance according to in vitro tests.

TABLE 1

Comparison of resistance to various antibiotics of strains of Shigella flexneri *isolated in different years*

Antibiotic	Year	Use of antibiotic in the hospital at the time	No. of strains tested	% resistant to 10 μg/ml or more
Tetracycline	1953	Lim.	31	0
	1955	Ext.	26	19
	1956	Ext.	41	34
	1957	Ext.	33	39
Chloramphenicol	1953	Lim.	31	0
	1955	Ext.	26	8
	1956	Ext.	41	7
	1957	Ext.	33	3
Streptomycin	1953	Ext.	31	74
	1955	Lim.	22	50
	1956	Lim.	18	39
	1957	Lim.	33	39

TABLE 2

Susceptibility of strains of Shigella flexneri *isolated during the years 1955–1957 to various concentrations of three different antibiotics*

Antibiotic	No. of strains tested	Per cent of strains inhibited by indicated concentration of antibiotic (μg/ml)						
		0.6	1.25	2.5	5	0	50	>50
Tetracycline	100	61	2	1	3	1	2	30
Chloramphenicol	100	75	12	4	2	1	0	6
Streptomycin	73	0	0	3	42	12	1	41

TABLE 3

Comparison of resistance to various antibiotics of strains of different serotypes of Shigella flexneri *isolated during the years of 1955 to 1957*

Serotype	Strains tested	% of strains resistant to 10 μg/ml or more of indicated antibiotic			
		Tetracycline	Chloramphenicol	Streptomycin	
				Strains tested	% resistant
S. flexneri 2a	54	34	2	37	41
S. flexneri 4	13	38	15	13	38
S. flexneri 6	12	42	0	10	60
S. flexneri 1b	8	28	28	7	57
S. flexneri 3	7	14	0	4	25
S. flexneri 1a	6	14	0	2	0
All types	100	32	6	73	42

A similar phenomenon of appearence of resistant strains was observed in the case of chloramphenicol, though it was not as striking as in the case of tetracycline.

With regard to streptomycin, an opposite change occurred. During the year of 1953 in which the substance was widely in use, 74% of resistant strains were found. However, the proportion decreased considerably during the following years (39% in 1957). It is to be noted that the use of the antibiotic became more limited after 1953.

The data shown in Table 2 show that the concentrations of tetracycline and chloramphenicol required to inhibit the growth of most of the strains of *Shigella* tested were smaller than those required of streptomycin. In the case of tetracycline, concentrations of 2.5 μg/ml or less were sufficient to inhibit 64 of 68 susceptible strains. With chloramphenicol, 91 of 94 susceptible strains were also inhibited with similar concentrations of the antibiotic. With streptomycin, on the other hand, only 3 of 32 susceptible strains were inhibited with this amount. Most of the strains considered as susceptible to this antibiotic required concentrations of 5 to 10 μg/ml of the substance, to be inhibited.

The data shown in Table 3 indicate that among the various serotypes of *Shigella flexneri*

that were tested there was not a significant difference in susceptibility to tetracycline, while with chloramphenicol and streptomycin, certain serotypes were more susceptible than others. Serotypes 1b and 4 were more resistant to chloramphenicol than the others, while more strains belonging to serotypes 6 and 1b were found resistant to streptomycin.

SUMMARY

The action of tetracycline, oxytetracycline, chlortetracycline, chloramphenicol and streptomycin, was studied on 131 strains of *Shigella flexneri* isolated during the years 1953, 1955, 1956, and 1957.

None of the 31 strains of *Shigella flexneri* tested in 1953 was resistant to tetracyclines. Among the strains isolated during the years 1955, 1956, and 1957, 19%, 34%, and 39%, respectively, were resistant to these antibiotics. The possible relationship between the emergence of the resistant strains and the extensive use of tetracyclines during the period of study is discussed.

A similar phenomenon was observed in the case of chloramphenicol, though not as striking as in the case of tetracycline. With streptomycin the opposite situation occurred.

The concentrations of tetracyclines and chloramphenicol required to inhibit growth of most of the susceptible strains of *Shigella* tested was smaller than that required with streptomycin.

Among the various serotypes of *Shigella flexneri* studied there was not a significant difference in resistance to tetracyclines, while with chloramphenicol, and streptomycin there were certain serotypes which were more resistant than others.

ACKNOWLEDGMENTS

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SIZE AS A CRITERION OF SPECIES IN THE HUMAN INTESTINAL AMEBAE

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In a recent paper,[1] we have shown that it is possible to use reproductive isolation in respect of size as a criterion of species in amebae. The present paper is an expansion of the preliminary report.

It is possible on nuclear structure to differentiate five different species of amebae inhabiting the human alimentary canal. These are *Entamoeba histolytica, Entamoeba coli, Endolimax nana, Iodamoeba bütschlii* and *Dientamoeba fragilis*. In view of its pathogenicity, it is of importance to differentiate *E. histolytica* from the remainder, but this species itself has been reported as including forms which vary considerably in size, feeding habits and pathogenicity. There is much controversy as to whether there are more than one species included under the name *E. histolytica*, or whether the differences reported are subspecies or possibly merely reflections of environmental difference.

In a previous paper,[2] it had been shown that *E. histolytica* can be successfully grown from human liver pus after suitable treatment and the provision of appropriate bacterial associates. The effect of different associates on amebae of similar and apparently uniform origin was then investigated. Amebae from three different liver abscesses *A*, *L*, and *D*†, were each cultured, on the one hand with a single organism, *Clostridium welchii*, Stock No. *68*, and on the other with a bacterial complex *m* previously isolated from a culture of *E. histolytica* derived from the stool of an African patient suffering from acute amebic dysentery. Measurements of the diameters of groups (44) of live, rounded-up trophozoites from these cultures yielded very different means for the lines with *Cl. welchii* (*68*) and those with the *m* complex (Table 1). It will be seen that there is a highly significant difference in mean size between the amebae associated with the two different bacterial associates.

To confirm that the difference was environmental and not genetic, two of the above lines with the same origin, *Lm* and *L68*, were cultured together in the same tube. This resulted in a population whose distribution was unimodal and the mean of which, 16.9μ (s.d. 2.3) lies between those of the two parent populations. These three populations are illustrated in Figure 1. This shows that *Cl. welchii*, the *m* complex, and the *m* complex plus *Cl. welchii*, each created different cultural environments which modified the size of the single, basic stock of amebae. No genetic change thus appears to have occurred in the amebae involved. The differences between the mean sizes of the three lines with *Cl. welchii* (and similarly, with the *m* complex) could be due to differences between the amebae, but, subsequent work indicates that it is probable that minor environmental differences between tubes are responsible.

Having established that the size of *E. histolytica* can be varied significantly by a change of bacterial associate, the next problem was to discover whether all size differences encountered between different populations of amebae with *'histolytica'* morphology were in fact due to environmental factors. We were fortunate at this stage of our investigation to find a patient with a stool containing what appeared to be two different size groups of *E. histolytica* (line *Zz*). The material was stained and both large and small types were shown to have the typical nuclear structure of *E. histolytica*. Measurements of the diameters of groups of 100 random, live, rounded-up amebae, at various intervals up to 2 months in culture, always gave a definite bimodal distribution curve. Figure 2 is the curve resulting from six such groups of measurements. The different heights of the two modes are, of course, due to unequal numbers of the two types. The approximate mean diameters of each of the two

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† Lines of amebae are designated by a capital letter, and the accompanying flora by either a lower-case letter where the flora is complex, or by a number when a single bacterial stock culture is used.

TABLE 1

The effect of the bacterial associates on the mean diameter of Cultured E. histolytica

Associate		Amebae		
		A	*L*	*D*
Cl. welchii (68)	mean	21.1μ	22.0μ	24.9μ
	s.d.	3.3	3.3	4.5
m complex	mean	13.6μ	15.4μ	13.2μ
	s.d.	2.0	2.4	2.1
Value of $\frac{\text{diff.}}{\text{s.d. diff.}}$		10	10	16

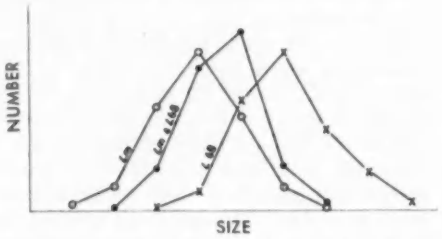

FIG. 1. Size distribution of live trophozoites of amebae in lines *Lm*, *L68* and *Lm & L68*

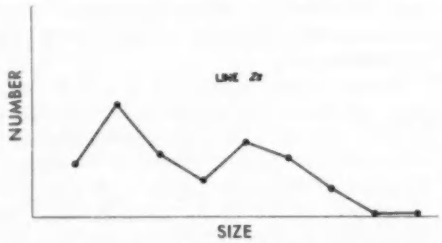

FIG. 2. Size distribution of live trophozoites of amebae in line *Zz*

groups of trophozoites are 7.5μ and 14.5μ, respectively.

The finding of a bimodal size distribution of *E. histolytica* in fecal specimens might possibly be interpreted as being due to the amebae having lived at different levels of the bowel. However, after coexistence in a test tube for 2 months, no such explanation is possible. It would seem quite clear that the size groups represent genetically different populations.

As a check on this conclusion, two lines of *E. histolytica* which had been for months in culture

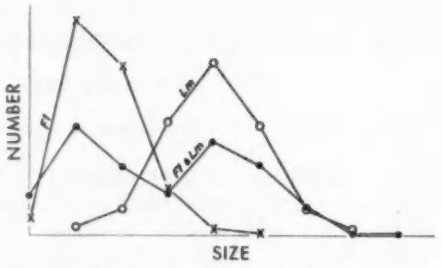

FIG. 3. Size distribution of live trophozoites of amebae in lines *Ff*, *Lm* and *Ff & Lm*

and had each retained its particular mean size, were mixed. The one culture *Ff*, had a mean size of 9.5μ and a standard deviation of 2.0; the other, *Lm*, had a mean of 11.4μ and standard deviation of 2.4. Each was associated with a mixed bacterial flora. Figure 3 shows the size distribution curves of the two cultures separately and also when mixed in a single tube. Here the mixed culture gave the clear bimodal curve shown for as long as the culture was kept. These results thus confirm the observations with *Zz* that, with regard to size, some populations with *'histolytica'* morphology are genetically different.

Because of the possible bearing of these results on the question of the pathogenicity of *E. histolytica*, it was decided to mix in culture *Ff* with a line designated *Bb*. The mean diameter of *Bb* is 19.2μ and its standard deviation is 3.1. This line, unlike *Lm* in which the amebae were from a liver abscess, was derived from a person who had carried the amebae for a long time and had at no time shown any symptoms attributable to the parasite. This mixture of *Bb* and *Ff* gave a bimodal curve (Fig. 4) similar to the one found with the *Ff-Lm* mixture, but the proportion of *B* amebae was relatively small.

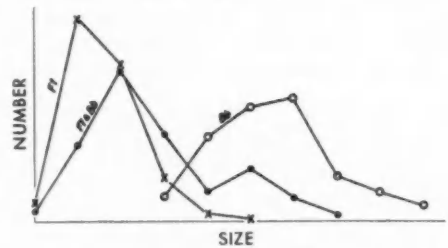

FIG. 4. Size distribution of live trophozoites of amebae in lines *Ff*, *Bb* and *Ff & Bb*

FIG. 5. Size distribution of live trophozoites of amebae in lines *Ff*, *L68* and *Ff* & *L68*

Amongst other mixtures tried, that of *Ff* plus *L68* is worthy of mention in that the vast majority of the amebae in the mixed culture were obviously *F* and only a few were *L*. It would seem that in the presence of the *f* flora plus the *68*, the *F* amebae thrived but the *L* amebae were reduced in size and number. Figure 5 shows the curve actually found with *Ff* and *L68*. In the presence of *f* and *m* flora (Fig. 3) *L* amebae were not suppressed. (The mathematical proof that the terminal part of the curve *Ff* plus *L68* represents the *L* amebae will be given with other mathematical considerations in a paper in preparation.)

As different single species and combinations of associates influence the size of amebae, it would appear that the only way to compare amebae of different origins is to place them in culture with the same associates. This can be done by adding both types of amebae with all their associates to a single tube as described above, but it is possible here that the presence of the "other" ameba is playing some part. Therefore, the problem was approached from another aspect.

The bacterial associates of morphologically different amebae were separated from the protozoa by light centrifugation of a shaken culture. After checking by culture that no amebae had been carried over, the flora of one line was then added to the other, and vice versa. Both lines were then grown with the composite floras of the two. The new sizes of the two lines were then computed and compared with the original and also with a mixture of both lines plus their floras in a single tube.

Table 2 gives a comparison of the sizes of *E. histolytica* (*L68*) and *E. coli* (*Rr*) when cultured in this way. It will be seen that the *r* flora, which is a complex, has drastically reduced the size of

TABLE 2

The effect on the mean diameter of the amebae in Rr *and* L68 *of adding the reciprocal bacterial flora*

Rr:	Mean—21.2μ; S.D. 3.6
Rr plus *68*:	Mean—21.7μ; S.D. 2.5
L68:	Mean—22.0μ; S.D. 3.3
L68 plus *r*:	Mean—13.8μ; S.D. 4.0

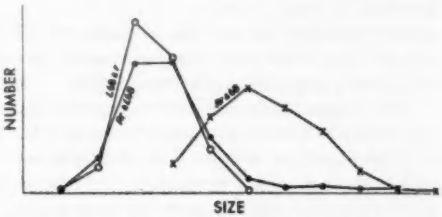

FIG. 6. Size distribution of live trophozoites of amebae in line *L68* & *r*, *Rr* & *68* and *L68* & *Rr*

amebae in *L68*. On the other hand, the addition of *68*, which is a single species *Cl. welchii*, to the amebae in *Rr* has had little or no effect on the size. In the mixture of *L68* and *Rr* in a single tube (Fig. 6) it appears that the *R* amebae (*E. coli*) have been swamped by the *L*, (*E. histolytica*). The presence of the *R* amebae in the culture is, however, proved by the extension of the terminal part of the curve, *L68* plus *Rr*, and is confirmed by morphological study. The size distribution of *L* seems to be about the same as in the *L68* plus *r* flora and it seems probable that the size of the *R* amebae is similar to that of the amebae in the *Rr* plus *68* culture.

This experiment thus shows that, in this particular mixture at least, *E. histolytica* and *E. coli* remain distinct and can be detected by the method used. Further, it confirms the size changes which are possible by changing the bacterial associates. Where mixtures show apparent fusion, it is essential to do measurements of fairly large numbers of individuals to rule out swamping effects.

DISCUSSION

There is a mass of literature on the subject of the size of the amebae known as *E. histolytica* which has been well reviewed by Hoare.[3] Perhaps the most significant paper reviewed is that by Sapero *et al.*,[4] in which the authors showed that a size-distribution curve derived from the measurement of some 7,500 cysts from 320 cases

was bimodal. This they took to indicate that there are two races of *E. histolytica*. Although 6.3% of the cases harbored both races, there remains the possibility that the size differences found may have been determined by the uncontrolled environmental factors *e.g.*, site and associates in the bowel. This criticism applies to much of the other work reviewed by Hoare.[3] Recently, however, Burrows[5] advanced evidence from nuclear morphology and the ratio of nuclear to overall diameter, that the large and small forms should be recognized as different species.

The present study has shown that the mean size of populations of *E. histolytica* can be varied considerably by a change in the bacterial associates. Though the full range of variation for this species and for other human parasitic amebae must still be worked out, it is obvious that random measurement of amebae without control of the environment is apt to be misleading. However, if amebae of two different origins, which, when grown separately, produce unimodal size distribution curves, are grown together in the same environment and over several generations continuously produce a bimodal curve, the size differences found are presumably genetic. This is the result that was obtained in the experiments with certain small and large amebae (*E. histolytica*) described in this paper.

In his review, Hoare[3] felt that the overlap in size between large and small *E. histolytica* precluded their separation into two species. When one now takes into consideration the morphological differences indicated by Burrows,[5] the segregation in the same environment reported here, and the generally accepted fact that the small ameba always lives in the host as a commensal, there can no longer be any doubt that the two forms merit specific separation. One must therefore conclude that there are two species of *Entamoeba* of similar morphology.[5] The larger of these amebae should be known as *E. histolytica* and the smaller as *E. hartmanni*.

Though it has its place in the differentiation of strains of amebae under cultivation, the technique described here is not practical for routine use. Similarly, the criteria described by Burrows[5] are rather complex for the average laboratory. However, because *E. hartmanni* is almost certainly nonpathogenic, some simple and accurate method of distinguishing the above two amebae is highly desirable. Until such a technique is devised, it might therefore be as well to revert to the practical criterion used by many in the past, and to regard populations of amebae with a mean cyst diameter smaller than 10μ as belonging to the species *E. hartmanni* and those with a mean cyst diameter above 10μ to *E. histolytica*. For the trophozoites, 12μ might be used as the dividing line. This will, of course, not differentiate between the commensal phase[3] and the invasive form of *E. histolytica*. The latter can at present be identified morphologically only by the presence of ingested red blood cells.

SUMMARY

1. The influence of bacterial associates on the size of human intestinal amebae in culture is shown.

2. The use of size-distribution curves to distinguish amebae in controlled environments is described.

3. Evidence of a genetic size difference between *E. histolytica* and *E. hartmanni* is presented.

4. It is suggested that, until an accurate, practical method for separation is devised, a mean size of 10μ for cysts and 12μ for trophozoites (a criterion often suggested in the past), be used as the dividing line between the larger species, *E. histolytica* and the smaller, *E. hartmanni*.

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THE HEMATOLOGICAL FINDINGS, SERUM PROTEIN PATTERN AND LIVER FUNCTION TESTS IN ACUTE AMEBIC DYSENTERY AND AMEBIC LIVER ABSCESS*

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For the most part liver function tests have been undertaken in amebiasis either as a diagnostic aid in hepatic amebiasis, or to estimate hepatic dysfunction in intestinal amebiasis. In many observations the tests have been fragmentary, while others have drawn no distinction between amebic hepatitis and liver abscess. Commonly there is a lack of controls in assessing both the diagnosis and therapeutic response of these cases. It is not surprising, therefore, that the literature shows wide differences of opinion on the effect of amebiasis on liver function and the serum protein pattern.

Most reports emphasize that changes in liver function are slight and inconsistent in hepatic amebiasis, although serum alkaline phosphatase activity and bromsulphalein retention are sometimes increased.[4, 5, 7, 8a, 11, 13, 23, 25, 27, 28, 31, 32] Some comment that the physical findings are more marked than laboratory evidence of hepatic dysfunction, in contrast to other forms of liver disease such as infectious hepatitis. Kean[16] observes that "it appears to be the consensus that approximately 50 per cent of individuals with diffuse amebic hepatitis will have 1 or more abnormal liver function tests."

However, others find that liver function is disturbed to a much greater extent in hepatic amebiasis. De Vries *et al.*,[10] report hyperglobulinemia, and, less often, hypoalbuminemia in liver abscess. Coiurault *et al.*,[8] found similar changes with a selective increase in gamma globulin in 6 Indo-Chinese patients. They also report raised $ZnSO_4$ turbidity and positive cephalin cholesterol tests. Coumel *et al.*,[9] found evidence of liver dysfunction in all of 37 cases of chronic amebiasis, amebic hepatitis and liver abscess, the cephalin cholesterol being one of the tests most frequently abnormal. Although liver function was more disturbed in those cases with clinical signs of hepatic involvement, they present evidence of liver dysfunction in intestinal amebiasis. This aspect has been emphasized by several workers,[6, 21, 26] Others [1, 2, 22] place great reliance on abnormal liver function tests, particularly the cephalin cholesterol, in the diagnosis and therapeutic assessment of hepatitis associated with chronic intestinal amebiasis.

In Durban amebiasis is common and often severe in the African. The following investigation was undertaken to determine and compare changes in the serum proteins and liver function tests in acute amebic dysentery and amebic liver abscess.

MATERIAL

Forty-nine African male patients with acute amebic dysentery, and 31 with uncomplicated amebic liver abscess were investigated on the day of admission and on completion of treatment 27 to 30 days later. Follow up studies were conducted for a further 3 months. All those with amebic dysentery had dysenteric symptoms, rectal ulceration and trophozoites of *E. histolytica* in their stools. All the cases of liver abscess were proven by aspiration of characteristic pus. In neither group was coexisting disease present.

As a control 50 healthy African male laborers who attended the Casualty Department with minor cuts and abrasions were investigated. All lived in Cato Manor, an African slum suburb from which are derived nearly all of our admissions with amebiasis. They shared the same poor socioeconomic background as our patients. The findings in this group have already been fully discussed.[24]

METHODS

Blood was collected between 9 and 10 a.m., and examined on the same day, the following

* This investigation was supported in part by a grant from the Council for Scientific and Industrial Research.

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TABLE 1

Hb (G/100 ml), wbc (cu mm), ESR (mm/1 hr), PCV (%) and mean corpuscular hemoglobin concentration (%) (MCHC) in amebic dysentery and amebic liver abscess

	Amebic dysentery		Liver abscess		African controls
	O/A	O/D	O/A	O/D	
Hb(G/100 ml) mean	15.8	16.0	12.5	15.2	16.0
Percentage with Hb < 14.5 G/100 ml	18	14	71	22	4
wbc/cu mm mean	13,100	9,700	13,900	9,200	—
Percentage with wbc > 10,000/cu mm	73	31	74	28	16
ESR mm/1 hr mean	22	19	43	28	18
Percentage with ESR > 9 mm/1 hr	80	67	87	78	78
PCV (%) mean	47	48	38	45	47.3
MCHC (%) mean	33.6	33.3	32.9	33.8	33.9

investigation being performed in all cases: Hemoglobin (Hb) read as oxyhemoglobin in a colorimeter; white cell count (wbc); erythrocyte sedimentation rate (ESR) by the Wintrobe method; and packed cell volume (PCV).

The serum bilirubin (normal range 0.1–0.8 mg./100 ml serum) and the alkaline phosphatase (normal range 3–13 King Armstrong units) were estimated by the methods of King and Wootton,[17, 18] and cephalin cholesterol flocculation by the method of Hanger[14] (normal range up to and including 2+ at 48 hours). The zinc sulfate turbidity test of Kunkel[19] was read visually, using Kingsbury sulfosalicylic acid standards, (normal range 0–4 units[20]. Comparison with $BaSO_4$ standards read colorimetrically showed that the latter gave results in units almost exactly double that of the visual technique.

Total serum protein, albumin and globulin were estimated by the method described by Weichselbaum,[20] the results being read in a colorimeter, using a green filter.

Paper electrophoresis was performed in a horizontal bath, using barbitone buffer, pH 8.6, and platinum electrodes. Serum (0.025 ml) was applied to 3-cm-wide strips of Whatman No. 1 filter paper. After running the strips in a current of 2 milliamps per strip for 18 hours, and drying and staining with 1% bromophenol blue in a 95% ethanol solution saturated with $MgCl_2$ for 5 minutes, the strips were washed successively with distilled water, dioxan and ether. They were finally dried in a hot air oven at 105°C for 15 minutes. The stained strips were then immersed in liquid paraffin at 100°C for 10 minutes, and scanned with a commercial photoelectric densitometer. The areas of the individual curves in the resulting graphs were measured and the percentage concentrations of the various protein fractions computed. The resulting percentages were not multiplied by any coefficient. The total protein obtained by the Weichselbaum technique was used to convert the percentage values of the protein fractions to absolute values in g/100 ml. All sera were examined in duplicate, and a difference of more than 2% between the same protein fractions was not accepted. Standardization was made as rigorous as possible, and all estimations were made personally.*

RESULTS

Hematology. Table 1 indicates that in acute amebic dysentery the Hb and ESR were not significantly disturbed, whereas in liver abscess the mean Hb was 12.5 g/100 ml, and the ESR, 45 mm/1 hr. Three quarters of these cases had a normochromic anemia. The Hb returned to normal, and the ESR was much reduced following treatment. In both the dysenteric cases and liver abscess there was a leukocytosis of the same order (mean 13,000 wbc/cu mm) in three quarters of the patients.

Serum Proteins. The results obtained by chemical fractionation and paper electrophoresis shown in Table 2 are similar.

Total protein in acute amebic dysentery is significantly lower than in either liver abscess or the control group ($P < 0.1\%$). This is due to a reduction of serum albumin without alteration of the total globulin. Following treatment the albumin rises to the average range, producing a rise in total protein to the level of the control group.

Despite a reduction of serum albumin below that of the dysenteric patients, total globulin in liver abscess is increased so that total protein remains within the usual range. Total protein rises after treatment by an increase in albumin without a corresponding fall in total globulin; but the albumin fraction remains lower than that of the control group. All these changes are statistically significant ($P < 0.1\%$).

The following changes in the globulin fractions

* Statistical analysis was that of Student's t-distribution for testing the significance of difference between means.

TABLE 2

Mean values, range and SD of serum proteins in G/100 ml by chemical fractionation and paper electrophoresis in acute amebic dysentery and amebic liver abscess

	Amebic dysentery		Liver abscess		African controls
	O/A	O/D	O/A	O/D	
Chemical fract.					
Total protein					
mean	6.52	7.20	7.10	7.56	7.14
range	4.48–8.15	5.95–8.40	5.60–8.99	6.16–9.65	6.20–8.26
S.D.*	.81	.62	.90	.69	.42
Ser. albumin					
mean	2.57	3.12	1.96	2.97	3.23
range	1.11–3.57	2.02–4.14	1.22–3.30	2.19–3.66	2.61–3.91
S.D.	.54	.47	.50	.38	.29
Ser. globulin					
mean	3.95	4.08	5.14	4.59	3.91
range	3.00–5.53	3.10–5.06	4.14–7.06	3.47–6.43	3.05–4.78
S.D.	.56	.50	.57	.38	.37
A/G ratio	.65:1	.76:1	.38:1	.65:1	.83:1
Paper electrophoresis					
Ser. albumin					
mean	2.83	3.46	2.32	3.40	3.53
range	1.56–3.66	2.48–4.34	1.52–3.81	2.93–4.05	3.04–4.32
S.D.	.48	.42	.54	.31	.26
Ser. globulin					
mean	3.69	3.74	4.78	4.16	3.61
range	2.87–4.88	2.80–4.58	3.74–6.82	3.07–6.12	2.94–4.47
S.D.	.53	.43	.79	.63	.31
A/G ratio	.76:1	.93:1	.49:1	.83:1	.98:1

* S.D. = Standard deviation.

TABLE 3

Mean values, range and SD of serum globulin fractions in G/100 ml by paper electrophoresis in acute amebic dysentery and amebic liver abscess

	Amebic dysentery		Liver abscess		African controls
	O/A	O/D	O/A	O/D	
Alpha$_1$					
mean	.38	.31	.48	.31	.33
range	.20–.56	.20–.48	.31–.73	.19–.48	.23–.41
S.D.	.09	.07	.10	.07	.04
Alpha$_2$					
mean	.90	.77	1.08	.76	.73
range	.57–1.35	.55–1.22	.65–1.57	.48–.99	.57–.96
S.D.	.18	.12	.20	.14	.10
Beta					
mean	.80	.90	.85	.91	.93
range	.58–1.06	.55–1.12	.61–1.14	.58–1.48	.66–1.26
S.D.	.13	.11	.13	.18	.11
Gamma					
mean	1.61	1.76	2.37	2.10	1.62
range	1.02–2.29	1.02–2.45	1.40–3.99	1.39–3.99	1.13–2.36
S.D.	.33	.35	.73	.53	.22

TABLE 4

Mean values of alkaline phosphatase and zinc sulfate turbidity tests in acute amebic dysentery and liver abscess

	Amebic dysentery		Liver abscess		African controls
	O/A	O/D	O/A	O/D	
Alkaline phosphatase (K.A. units)	9.1	9.3	13.6	11.5	9.3
Zinc sulfate turbidity	5.9	6.8	14.2	12.8	6.3

are shown in Table 3: a) In both amebic dysentery and liver abscess the alpha fractions are significantly raised ($P < 0.1\%$), being higher in liver abscess. Following treatment they fall to the level of the control group. b) The beta globulins in both conditions are somewhat reduced, returning to the average African level following treatment. c) The gamma globulin in amebic dysentery is not significantly altered, whereas in liver abscess it is markedly and significantly raised ($P < 0.1\%$); largely accounting for the rise in total globulin. There is only a slight fall at the end of 27 to 30 days of treatment.

Follow-up studies showed that in liver abscess the serum albumin only reached the level of the controls 1 month after discharge. Although falling slightly, the gamma, and with it the total globulin, remained significantly elevated ($P < 0.1\%$), and did not fall to the control level until 3 months after discharge. In the dysenteric patients the serum proteins remained within the range of the controls throughout the follow-up period.

Liver Function Tests. Reference to Table 4 shows that the mean alkaline phosphatase and $ZnSO_4$ turbidity in amebic dysentery do not differ from those of the control group, while in liver abscess both are clearly raised, although there is a slight reduction on completion of treatment. The mean $ZnSO_4$ turbidity is above a strict European normal in all groups. One third of those with liver abscess had a raised alkaline phosphatase, and almost all an elevated $ZnSO_4$ turbidity.

The cephalin cholesterol test was not consistent, tending to become positive following treatment in both groups, although less commonly than in the controls. The serum bilirubin was normal in all cases.

Follow-up studies showed that in the dysenteric cases, the liver function tests remained within the range of the control group. In liver abscess the alkaline phosphatase returned to normal within 1 month of discharge. The $ZnSO_4$ turbidity ran parallel with the gamma-globulin level and returned to the control level after 3 months.

DISCUSSION

In Durban Africans with acute amebic dysentery the Hb, ESR, serum bilirubin, alkaline phosphatase and $ZnSO_4$ turbidity are seldom disturbed, but leukocytosis is common. In this investigation, the cephalin cholesterol test was more frequently positive in the control groups than in the patients. Anderson *et al.*,[3] report similar results and it would appear that little reliance can be placed on this test in the African. The mean gamma globulin is not elevated above the usual African level, and the total globulin is undisturbed. Extensive necrosis in the bowel wall due to the severe intestinal ulceration characteristic of the disease in the Durban African is sufficient to explain the increase in alpha globulins which return to normal following treatment. Reduction of the serum albumin might be due to hepatic dysfunction, but lack of absorption with increased protein loss is a more likely cause in severe dysentery. In all the dysenteric patients symptoms were rapidly controlled. The serum albumin rose to the control mean on completion of treatment and remained at this level during follow-up, indicating that cessation of dysentery combined with hospital diet quickly corrected the hypoalbuminemia. Apart from reduction in serum albumin and elevation of the alpha globulins, which can be explained by bowel disturbance, 98% of these cases have liver function tests within the normal African range.

In liver abscess the findings are clearly different. Anemia, leukocytosis and an elevated ESR are common features. The latter has been stressed by Blanc[4] and others as an index of hepatic involvement. In this series the serum bilirubin was not raised. We have found that jaundice is an uncommon finding in the African with liver abscess. When present, it is usually associated with a large abscess and is obstructive in type. The incidence is much less than that of 15% found by Sodeman and Lewis[29] in hepatic amebiasis. The alkaline phosphatase is inconsistent, being elevated in about a third of the cases, the mean value lying just above the normal range, although it is within the control range in all except one patient. The shortcomings of the cephalin cholesterol test in the African have already been noted. The $ZnSO_4$ turbidity is markedly elevated, and can be correlated with the increased gamma globulin. Elevation of the alpha and gamma fractions result in a raised total globulin. Neither of these changes in the globulins necessarily indicates liver disease, as elevation of the alpha fractions commonly occurs in inflammatory lesions with tissue breakdown, while gamma-globulin increase is common in chronic infections.[12, 13] As none of these cases had dysenteric symptoms it is unlikely that either lack of absorption with increased protein loss or dietary protein deficiency is responsible for the marked reduction of serum albumin in liver abscess. The control level is not reached until 1 month after discharge and the full serum-protein pattern does not return to the average African range until 3 months after treatment, indicating a slower return to the African normal due to a more profound disturbance of protein metabolism such as might be found in liver disease. Certainly, the findings in most cases are indistinguishable from those due to hepatic dysfunction.

Reduction of serum albumin and elevation of the gamma globulin with associated abnormal liver function tests are common in the symptomless African. Although the cause of the gamma-globulin elevation is obscure, it is likely that the reduced serum albumin is predominantly dietary in origin.[24] Similar changes are reported in malnourished races throughout the world, particularly in tropical regions. These disturbances must be borne in mind when ascribing changes to specific diseases. Moreover, it is probable that these subjects have less reserve of liver function than well-nourished Europeans, and may therefore show earlier or more marked disturbance of liver function in diseases affecting the liver. In addition, such subjects tend to wait until the disease is gross before seeking medical attention. These factors may be of importance when comparing the findings in Durban with those reported in Europeans, and do not necessarily contradict the many reports of only slight disturbance of liver function in Europeans with liver abscess. However, the latter reports seem incompatible with those which emphasize the value of liver-function tests in the diagnosis of hepatitis associated with

TABLE 5

Summary of changes in acute amebic dysentery and amebic liver abscess

	Amebic dysentery	Liver abscess
Anemia	uncommon	common
Leukocytosis	common	common
Elevated ESR	uncommon	common
Total proteins	low	normal
Serum albumin	low	low
Serum total globulin	not raised	raised
Alpha globulins	raised	raised
B. globulins	slightly low	slightly low
Gamma globulin	not raised	raised
Alkaline phosphatase	not raised	sometimes raised
Zinc sulfate turbidity	not raised	raised

chronic amebiasis. In the Durban African, amebic hepatitis (as distinct from amebic liver abscess) and chronic amebiasis are rarely diagnosed, whereas amebic dysentery and liver abscess are extremely common. In amebic dysentery there is little evidence of hepatic dysfunction, in contrast to liver abscess in which the laboratory findings are indistinguishable from those of disordered liver function.

Table 5 compares, in summary form, the changes found in acute amebic dysentery and amebic liver abscess.

SUMMARY

The literature dealing with liver function tests and changes in the serum proteins in amebiasis is reviewed and the results of hematological investigations, liver function tests, serum proteins and electrophoretic protein patterns in 49 cases of acute amebic dysentery and 31 of amebic liver abscess, investigated on admission, on discharge 27 to 30 days later and during follow-up, are reported. These findings are compared with each other and with 50 African control subjects.

It is concluded that in amebic dysentery the findings are compatible with disturbance of bowel function, whereas in liver abscess the changes are indistinguishable from those due to hepatic dysfunction.

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THE SERUM PROTEIN PATTERN, LIVER FUNCTION TESTS AND HEMATOLOGICAL FINDINGS IN THE DIFFERENTIAL DIAGNOSIS OF AMEBIC LIVER ABSCESS*

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Differences in the serum protein pattern and liver function tests between the African and European are well described, and the findings in the Natal African have been shown to conform with those reported elsewhere.[19] The changes in amebic liver abscess have been described,[20] but the diagnostic value of these findings depends on their specificity. In this region the conditions which commonly require to be differentiated from liver abscess are cirrhosis of the liver, primary carcinoma of the liver and right basal pneumonia. The following study was undertaken to compare the laboratory findings in these diseases with those in liver abscess.

MATERIAL

Three groups of African male patients were investigated, comprising 10 cases each of cirrhosis of the liver, primary carcinoma of the liver and right basal pneumonia. All could clinically have been confused with amebic liver abscess. Liver biopsy confirmed the diagnosis in all the cases of cirrhosis and primary hepatic carcinoma. Those with lobar pneumonia had radiological evidence of right basal consolidation and showed a rapid and complete response to penicillin.

Thirty-one patients with amebic liver abscess, proven by aspiration of characteristic pus, and a control group of 50 healthy African male laborers were also investigated. The findings in these groups have already been discussed in detail.[19, 20]

METHODS

These were identical to those described in a previous paper.[20]

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RESULTS

Hematology. Reference to Table 1 shows that a slight degree of anemia is frequent in all the conditions, although in liver abscess this is often more severe. Leukocytosis is not common in cirrhosis, but the white cell count is not helpful in differentiating between lobar pneumonia, liver abscess and primary carcinoma of the liver. Although elevation of the sedimentation rate is common in symptomless Africans the mean level is clearly raised in all the conditions.

Liver Function Tests. In primary carcinoma of the liver the alkaline phosphatase is almost always clearly raised (see Table 2). In both cirrhosis and liver abscess it is elevated in a third to a half of the cases, the mean level being somewhat higher in cirrhosis than in liver abscess. In lobar pneumonia it is not significantly affected. Although the $ZnSO_4$ turbidity is raised in the average African, in cirrhosis it is markedly elevated. It is commonly raised but to a lesser extent in primary carcinoma and liver abscess. It is only slightly affected in lobar pneumonia. In contrast to liver abscess the serum bilirubin is elevated in about half the cases of cirrhosis and primary carcinoma, and in a minority of those with lobar pneumonia. The cephalin cholesterol is difficult to assess as it is so commonly positive in the controls. However, it is frequently positive in both cirrhosis and primary carcinoma of the liver, and far less frequently in liver abscess and lobar pneumonia.

Serum Proteins. Both chemical fractionation and paper electrophoresis of the serum proteins show similar trends. Table 3 indicates that: a) in all the diseases the serum albumin is reduced significantly below that of the controls ($P < 0.1\%$). The reduction is most marked and of a similar order in cirrhosis, primary carcinoma and liver abscess. In lobar pneumonia the serum albumin is significantly higher than in the other diseases ($P < 0.1\%$), but is significantly lower than that

TABLE 1

Hb (g/100 ml), wbc (cu mm), ESR (mm/1 hr.) and mean corpuscular hemoglobin concentration (%) (MCHC)

	Cirrhosis	Primary carc. of the liver	Lobar pneumonia	Liver abscess	African controls
Hb mean	13.1	13.5	14.2	12.5	16.0
Percentage with Hb < 14.5 g/100 ml	90	70	60	71	4
wbc per cu mm mean	7,800	11,300	13,900	13,900	—
Percentage with wbc > 10,000 per cu mm	20	60	50	74	—
ESR mm/1 hr mean	42	40	33	45	18
Percentage with ESR > 9 mm/1 hr	90	90	90	87	78
MCHC mean	34.1	32.2	32.4	32.9	33.9

TABLE 2

Liver function tests

	Cirrhosis	Primary carc. of the liver	Lobar pneumonia	Liver abscess	African controls
Alkaline phosphatase (K.A. units) mean	17.2	37.5	9.6	13.6	9.3
Percentage > 13 units	50	90	10	37	6
Zinc Sulf. Turbidity, mean	23.1	16.1	7.4	14.2	6.3
Percentage > 4 units	100	80	80	97	84
Serum bilirubin, mean	1.3	1.9	0.7	—	0.4
Percentage > 1 mg/100 ml	40	50	10	0	0
Ceph. cholesterol percentage > ++/48 hr	80	70	20	12	44

TABLE 3

Mean values of serum proteins in g/100 ml by chemical fractionation and paper electrophoresis

	Cirrhosis	Primary carc. of the liver	Lobar pneumonia	Liver abscess	African controls
Chemical fractionation					
Total protein					
mean	7.40	6.94	6.74	7.10	7.14
range	5.83–9.42	5.91–7.85	5.90–7.85	5.60–8.99	6.20–8.26
S.D.*	.94	.63	.65	.90	.42
Serum albumin					
mean	2.02	1.95	2.47	1.96	3.23
range	1.17–3.08	1.15–2.74	1.90–2.85	1.22–3.30	2.61–3.91
S.D.	.66	.51	.29	.50	.29
Serum globulin					
mean	5.38	4.99	4.27	5.14	3.91
range	4.36–6.66	3.17–5.89	3.66–5.23	4.14–7.06	3.05–4.78
A/G ratio	.38:1	.39:1	.58:1	.38:1	.83:1
Paper electrophoresis					
Serum albumin					
mean	2.57	2.38	2.75	2.32	3.53
range	1.39–3.50	1.77–3.17	2.19–3.09	1.52–3.81	3.04–4.32
S.D.	.74	.45	.35	.54	.26
Serum globulin					
mean	4.83	4.56	3.99	4.78	3.61
range	3.54–6.05	3.06–5.59	3.58–4.93	3.74–6.82	2.94–4.47
S.D.	.75	.77	.39	.79	.31
A/G ratio	.53:1	.52:1	.69:1	.49:1	.98:1

* S.D. = Standard deviation.

TABLE 4.

Mean values of serum globulin fractions in g/100 ml by paper electrophoresis

	Cirrhosis	Primary carc. of the liver	Lobar pneumonia	Liver abscess	African controls
Alpha$_1$					
mean	.27	.43	.47	.48	.33
range	.17–.44	.31–.51	.32–.62	.31–.73	.23–.41
S.D.	.08	.07	.10	.10	.04
Alpha$_2$					
mean	.61	.88	1.04	1.08	.73
range	.42–1.03	.60–1.16	.73–1.27	.65–1.57	.57–.96
S.D.	.17	.20	.17	.20	.10
Beta					
mean	.85	.91	.81	.85	.93
range	.55–1.38	.73–1.13	.69–.91	.61–1.14	.66–1.26
S.D.	.28	.13	.06	.13	.11
Gamma					
mean	3.10	2.34	1.67	2.37	1.62
range	2.13–3.97	1.32–3.74	1.18–2.49	1.40–3.99	1.13–2.36
S.D.	.65	.78	.41	.73	.22

of the controls ($P < 0.1\%$). b) Conversely, in all the diseases the total globulin is significantly elevated above that of the control group ($P < 0.1\%$), although in lobar pneumonia the elevation is significantly less than in cirrhosis, primary carcinoma and liver abscess ($P < 0.1\%$).

Table 4 indicates the following changes in the

globulin fractions: a) The alpha globulins in cirrhosis are significantly lower than in primary carcinoma, lobar pneumonia, liver abscess and the control group ($P < 0.1\%$). In primary carcinoma, lobar pneumonia and liver abscess they are significantly elevated above that of the controls ($P < 0.1\%$). However, the elevation is significantly less in primary carcinoma ($P < 0.1\%$). b) In lobar pneumonia the gamma globulin is not significantly disturbed, whereas in each of the other three conditions it is significantly elevated above that of the control group ($P < 0.1\%$), being highest in cirrhosis.

DISCUSSION

The changes in the blood picture, liver function tests and serum proteins in Europeans with cirrhosis of the liver are fully described.[3, 4, 7, 8, 11-17, 21, 25-27] Despite the differences between apparently healthy Africans and Europeans, it is evident that in African patients with clinical symptoms and signs of cirrhosis the laboratory findings conform to those described in Europeans.

Reports of the findings in primary carcinoma of the liver differ considerably. Most of the data are based on European subjects. Some note that elevation of the serum bilirubin and alkaline phosphatase are common.[5, 18, 23, 24] Others do not find these tests helpful.[2, 6, 9, 22] In a study of the changes in African subjects, Bersohn[1] found the mean level of the alkaline phosphatase to be higher in primary carcinoma than in cirrhosis. He also noted elevation of the $alpha_2$ and beta globulins, and a dissociation between the alkaline phosphatase and serum bilirubin levels in primary carcinoma.

Spellberg[24] states that liver-function tests in primary carcinoma are variable, and depend to a great extent on the presence of an underlying cirrhosis. If there is marked derangement of the "liver profile" the presence of cirrhosis is likely. My findings indicate that, although the mean values in primary carcinoma of the liver show a pattern of liver-function tests and serum proteins different from those in cirrhosis and liver abscess, there is a wide variation in the individual cases. In some instances the findings are similar to those in cirrhosis. In others there is comparatively little disturbance. No correlation could be found from liver biopsy and autopsy findings between either the type of carcinoma or the degree of accompanying cirrhosis with the liver-function tests and protein patterns of the individual cases. However, the alkaline phosphatase was clearly raised in nine of ten cases and tended to be considerably higher than in cirrhosis. The alpha globulins were elevated in seven cases. This was not found in cirrhosis, presumably as they depend on the amount of tissue destruction occurring within the carcinoma. The cephalin cholesterol, serum bilirubin, $ZnSO_4$ turbidity, albumin and globulin levels were not of assistance in differentiating the two conditions, although a negative cephalin cholesterol with a normal $ZnSO_4$ turbidity and gamma globulin favour primary carcinoma of the liver.

Sherlock[22] states that a leukocytosis of about 10,000 cells per cu mm is usually present in primary carcinoma as opposed to a leukopenia in cirrhosis, and the finding of a leukocytosis in cirrhosis should suggest a complication such as a primary liver-cell carcinoma. In the present series seven of ten cases of primary carcinoma had a leukocytosis compared with only two in cirrhosis. A leukocytosis was present in all the cases of primary carcinoma with raised alpha globulins.

In lobar pneumonia the globulins showed a distinct difference from those in liver disease on the one hand, and the African controls on the other. Nine showed elevation of the alpha globulins, such an increase being well described in lobar pneumonia.[3, 7, 8, 10] The gamma globulin was comparatively undisturbed. Apart from elevation of the serum bilirubin and a raised alkaline phosphatase in one case each, the liver function tests remained within the usual African range. This is in contrast to the findings of Zimmerman and Thomas[28] who found that liver function was widely and almost invariably impaired in American Negroes with pneumococcal pneumonia.

CONCLUSIONS

The diagnosis of amebic liver abscess is primarily clinical, although radiology and aspiration are of notable assistance. The place of laboratory investigations in the differentiation of this condition from cirrhosis, primary carcinoma of the liver and right basal pneumonia is small. However, comparison of the changes found in these four diseases indicate the following points which may be of some help occasionally:

1. Leukocytosis is uncommon in cirrhosis.

2. Elevation of the serum bilirubin is uncommon in liver abscess.

3. The alkaline phosphatase tends to be highest

and most consistently raised in primary carcinoma. It is rarely elevated in lobar pneumonia.

4. The cephalin cholesterol test is of little value in the African, but it is unlikely to be negative in cirrhosis.

5. The $ZnSO_4$ turbidity is consistently and most markedly raised in cirrhosis. It is almost always raised, but to a somewhat lesser extent in primary carcinoma and liver abscess. It is not significantly elevated above the average African range in lobar pneumonia.

6. The serum albumin is commonly reduced in all four conditions, but less markedly so in lobar pneumonia.

7. Conversely, the total globulin is commonly raised in all four conditions, but less markedly so in lobar pneumonia.

8. The alpha globulins are not raised in cirrhosis. They may be raised in primary carcinoma, and are very commonly elevated in liver abscess and lobar pneumonia.

9. Changes in the gamma globulin are paralleled by the $ZnSO_4$ turbidity; a marked elevation occurring in cirrhosis, a somewhat less marked rise in liver abscess and primary carcinoma and usually little change being found in lobar pneumonia.

SUMMARY

1. The results of the haematological investigations, liver function tests and electrophoretic serum protein patterns are presented in 10 patients, each with cirrhosis of the liver, primary carcinoma of the liver and right basal pneumonia.

2. These findings are compared with each other, and with those in 31 cases of amebic liver abscess and 50 African controls.

3. It is concluded that, though the results indicate certain differences between these conditions, their value in differential diagnosis is not great.

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TREATMENT OF AMERICAN DERMAL LEISHMANIASIS WITH A SPECIAL ORAL DOSAGE FORM OF TARTAR EMETIC

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INTRODUCTION

The status of dermal leishmaniasis on the Isthmus of Panamá has been reviewed in detail by Calero and Johnson[2] and by Langsjoen.[5] As they pointed out, the disease in this area has rarely been characterized by the nasal mucous-membrane involvement which is typical of *Leishmania brasiliensis* infections in other regions; only one such case had thus far been reported. The typical patient presents himself to the physician with one or more chronic ulcerations on the extremities and gives a history of clearing virgin forest, sowing, harvesting or hunting just prior to onset of the illness. In the absence of secondary infection, lesions on persons in this geographic location tend to spontaneous cure only after several months to a year or more in nonimmunes, leaving a smooth, glistening scar. In surveys of certain areas, many instances of this healed, cicatricial stage have been encountered. Reinfections are uncommon in persons who have once been cured of American dermal leishmaniasis, caused by *L. brasiliensis*, just as Sagher, *et al.*,[8] have shown that they are in persons cured of oriental sore due to *L. tropica*.

Various treatment measures have been employed and the antimonials stand high on the list of preferred agents. Stibophen administered by intramuscular injection is probably the most used of these compounds. But, in addition to the inconvenience of a series of injections, there is a limit to the effectiveness of stibophen in the opinion of some investigators and clinicians. For example, Carl M. Johnson (Personal communication, 1957) says: "We have had consistently poor results with stibophen. The lesions heal up nicely, but after a period of time a series of verrucous-like lesions develop around the periphery of the scar . . ."

In 1956, Peña Chavarria[7] reported from Costa Rica that a useful dosage form, especially in children with cutaneous leishmaniasis, was a solution of tartar emetic in water-plus-chloroform. The concentration used was 2 g of drug in 80 ml of liquid, or 1 mg per drop. Initial dose in children was one drop daily; adults were given 5 to 15. These dosages were gradually increased to tolerance, and maintained for as long as 3.5 months. Permanent cures were reported in 10 adults, 20 children.

It is the purpose of this brief report to discuss results obtained with a solid oral form of potassium antimony tartrate (tartar emetic) in three patients with cutaneous leishmaniasis.

MATERIAL AND METHOD

A special oral dosage form of tartar emetic, designed to provide prolonged absorption, was prepared by The Upjohn Company, Kalamazoo, Michigan, under Research Number 11,987-1. Single capsules assayed 23.7 mg of potassium antimony tartrate.

Tolerance studies were done in 30 volunteers. It was determined that 24 out of 25 adults (96%) could tolerate one capsule three or four times daily without undue subjective symptoms for periods varying from 12 to 45 days. However, even a single dose of two capsules would frequently cause nausea and occasionally vomiting in some of these same persons as well as in all five of the individuals who received only such single doses of two or more capsules.

It was next demonstrated unequivocally that the drug was absorbed from the gastrointestinal tract by doing quantitative analyses of 24-hour pooled urine specimens from volunteers given approximately equal gravimetric doses of metallic antimony in the oral capsule form, or as stibophen by the intramuscular route. Results obtained by using Maren's rhodamine B method[6] with Freedman's perchloric acid modification,[4] are shown in Table 1. The two patients who were given the single oral dose of tartar emetic in the special form, excreted antimony in the urine in concentrations similar to those of the patient who was given the intramuscular stibophen. The total weight of antimony excreted in the 48-hour

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TABLE 1

Quantitative studies of urinary antimony excretion in subjects given equal gravimetric single doses orally or intramuscularly (as stibophen)

Subject	Drug and dosage	Route	Collection period (*hrs*)	Mg Sb per L	Total mg Sb excreted
1. N.R.	136 mg. stibophen equal to 18.4 mg trivalent metallic Sb	i-m	0–24 24–48	0.310 0.490	1.160 1.570 2.730
2. I.C.	47.4 mg tartar emetic (Upjohn Co. 11,987-1) equal to 17.3 mg trivalent metallic Sb	PO	0–24 24–48	0.044 0.390	0.045 0.460 0.505
3. J.P.	47.4 mg tartar emetic (Upjohn Co. 11,987-1) equal to 17.3 mg trivalent metallic Sb	PO	0–24 24–48	1.330 0.024	1.100 0.044 1.144

urine samples of the patient receiving stibophen was greater than the amounts excreted in 48-hour urine samples of the patients administered the oral tartar emetic. As pointed out in the Discussion, these results would be expected.

Three patients with dermal leishmaniasis were then treated to see if amounts absorbed would be sufficient to exert a therapeutic effect (e.g., produce healing in a significantly shorter time than would have been expected without other measures specifically directed at the parasite).

RESULTS

The results are presented in the following three case reports, and in Table 2.

TABLE 2

Results in American dermal leishmaniasis treated with oral potassium antimony tartrate

Case No.	Age (*yrs*)	No. of ulcers	Total capsules	Days of treatment	Total g potassium antimony tartrate	Weeks elapsing until complete cure attained	Duration follow-up
1	23	2	52	18	1.23	4.5	No relapse 15 mo.
2	22	3	120	30	2.84	8	No relapse 17 mo.
3	21	1	72	24	1.71	9	No relapse 18 mo.

Case 1. J.P.A., a 23-year-old Panamanian male was hospitalized on January 31, 1957, because of a deep ulcer on the dorsum of the left hand and a superficial, secondarily infected, ulcer on the right ear lobe. About a month previously, he had noticed a small lesion on the dorsum of the left hand, hardly pin-point in size. With scratching, this enlarged rapidly until at the time of hospitalization there was an ulcer 3.5 cm in diameter, round and even. The margin was raised, hard, and slightly undermined. The center was red, granulating, and only slightly moist. The lesion on the ear-lobe had begun 3 weeks previously, and at the time of admission occupied several sq cm. It was crusted and purulent, but when cleaned presented a granulating surface with slightly raised margin. A general physical examination was otherwise normal. Routine blood, urine, stool, chest x-ray and electrocardiograph examinations were normal. Smears of tissue juice from the ulcer edges revealed objects morphologically similar to Donovan bodies, and the diagnosis of leishmaniasis was confirmed by a biopsy.

Tartar emetic capsules were started on February 5, 1957. Three capsules were taken daily at intervals and this was continued until February 22. For the first 10 days, healing was very slow. Then the lesions began to dry and diminish in size, so that after 17 days of treatment there was marked change. The medication was discontinued, but the patient was kept under close observation. Healing continued rapidly, and in 2 more weeks only a smooth, slightly pink scar remained. The patient was seen at intervals for more than 15 months after treatment, and there was no recurrence.

Case 2. J.A.H., a 22-year-old Panamanian farm laborer was hospitalized on February 15, 1957, with three ulcers: one on the right wrist, one on the right forearm, and one on the left lower leg. The lesion on the right wrist had begun 3 weeks previously, and the other two commenced a week later. All had appeared first as tiny papules, developed into vesicles, then rapidly enlarged and ulcerated as a result of scratching. They varied from 1 to 3 cm in diameter, had the typical raised, hard, undermined borders and granular red centers. In all three, some crusting and secondary infection was apparent. Physical examination was normal except for these skin lesions. Routine blood, urine, stool, chest x-ray and electrocardiograph examinations were normal. Tissue-juice preparations revealed objects morphologically similar to Donovan bodies, and the diagnosis of leishmaniasis was confirmed by biopsy.

On February 19, tartar emetic capsules were started, in the dosage of four capsules daily at intervals, and continued until March 3 (13 days). Improvement was very slow. On March 11, the same dosage was resumed and was continued until March 28. Healing during this time was more rapid. On further observation there was complete healing, leaving only smooth scars. In 17 months of posttreatment observation there has been no recurrence.

Case 3. S.B.S., a 21-year-old Panamanian female was hospitalized on February 25, 1957, with a large ulcer in the area of the right, inner maleolus. She stated that about 4 months previously she had had an insect bite which increased gradually in size in spite of many medications. At the time of admission to the hospital, the ulcer measured 7 cm in diameter. It had a thick, raised, hard border, and a red granulating center which was covered with a crust. Tissue-juice preparations revealed objects morphologically similar to Donovan bodies, and a biopsy from the edge of the ulcer confirmed the diagnosis of leishmaniasis.

Treatment with tartar emetic capsules was started on February 28, 1957, and continued in the dose of 3 capsules daily at intervals for 24 days. The lesion healed slowly at first, then more rapidly. Healing continued after the medication was discontinued, and at 2 months only a smooth, glistening, even scar remained. In 18 months of posttreatment observation, there has been no relapse.

The three patients tolerated the drug well, without evidence of toxicity, either clinical or as judged by urine and blood examinations. Electrocardiograms taken between 2 and 3 weeks after onset of treatment showed no changes from the pretreatment tracings.

DISCUSSION

The absorption and urinary excretion of antimony following oral administration of tartar emetic capsules has been demonstrated, and Table 1 shows that the metallic antimony content of urine in mg per L is comparable whether the antimony was administered orally or by intramuscular injection. Since the data of Bartter, *et al.*,[1] indicate that the blood level of antimony and the urinary excretion of antimony following intravenous administration of tartar emetic depend upon both the concentration of antimony and the time after administration, caution must be exercised in the interpretation and in the comparison of the per cent of the dose of antimony excreted after oral administration with that after intramuscular stibophen or intravenous tartar emetic; even though the same time interval (48 hours) is involved, and approximately the same dose of antimony. Since the concentration of metal in the blood following the administration of antimony orally would not be expected to reach anywhere near the levels attained following intramuscular or intravenous administration (due to the prolonged absorption of antimony attained with the type of oral medication employed), the urinary excretion of antimony in the 48-hour period would not be expected to be nearly as high following the oral as following the intravenous or intramuscular administration of antimony. This would be true even though the dose of antimony was the same by all routes and the same fraction of the dose was excreted in the feces following administration by each of these routes.

Although healing of the ulcers in the three cases of dermal leishmaniasis was not dramatic, it did occur in less than the average time required for healing without treatment. Langsjoen[5] reported healing times following stibophen ranging from 10 to 28 days in ten cases treated in the Panama area. Four promptly relapsed, requiring further treatment. As is shown in Table 2, 4.5, 8 and 9 weeks were required for complete healing in this small series. But no relapse was noted during follow-up periods of 15, 17 and 18 months. In all three, healing proceeded very slowly for about 10 days, thereafter more rapidly, and then con-

tinued into the cicatricial stage even after the capsules were discontinued. Because of the smallness of the series, generalizations are unwarranted. Treatment of additional cases by several clinicians is under way.

Presently employed doses of stibophen in schistosomasis suppress oviposition of the worms, sometimes temporarily and sometimes for more prolonged periods, but do not bring about a cure.[3] In addition, recent reports by Spingarn and Edelman[10] and by Schick, *et al.*,[9] suggest caution ln the use of stibophen because of frequent evidence of cardiac toxicity with the drug, and a significant, although small, mortality associated with its use. Therefore, new developments in antimony therapy are of interest. It would seem important to do further clinical and laboratory evaluation of the effectiveness of this oral tartar emetic dosage form, not only in leishmaniasis but in schistosomiasis and other diseases in which antimonials are effective. In doing such clinical trials, the potential toxicity of all antimony compounds demands that caution and prudence be excercised in the determination of dosage. We recommend that the dosage not exceed one capsule three times a day. It is possible that some individuals may experience vomiting or other unpleasant side effects even with this low amount; this would be a limiting factor in the use of this formulation as a therapeutic agent.

SUMMARY

A special form of oral potassium antimony tartrate (prepared by The Upjohn Company) is absorbed from the gastro-intestinal tract and antimony is excreted in the urine. Approximately 23 mg of this potassium antimony tartrate have been taken by a normal adult three or four times daily for as long as 45 days, with no evidence of significant toxicity.

In three instances American dermal leishmaniasis due to *L. brasiliensis* was successfully treated with this preparation.

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HEMAGGLUTINATION-INHIBITION AND NEUTRALIZATION TESTS FOR THE VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

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In the course of a survey for antibodies to different arthropod-borne viruses which is in progress at the Facultad de Medicina in Cali, Colombia, special emphasis has been given to the recognition of antibodies to the Venezuelan equine encephalomyelitis virus (VEE).

Infection with this agent has caused a human epidemic in Colombia.[11] The virus has also been isolated from febrile cases in the San Vicente de Chucurí area where serological evidence has been found of a high incidence of previous infections with this virus.[7] There is also proof that the VEE virus has attacked the human population in other localities in Colombia, including the Pacific coast, the Magdalena river valley and, almost certainly, the upper Cauca river valley.[12] The importance of this virus in other areas in the general region of northern South America was demonstrated by the two fatal human cases in Trinidad[6, 8] and the finding of antibodies to the agent among the population of that island.[5] In Brazil, near Belem, VEE virus isolations from man, sentinel monkeys and mosquitoes, and neutralizing antibodies in humans have been recently reported.[3]

The purpose of this paper is to present the comparative results of studies of 487 human sera by means of the hemagglutination inhibition (HI) and the neutralization (N) tests with the VEE virus.

MATERIALS AND METHODS

Sera. All specimens were kept frozen at −20°C in lusteroid tubes and thawed when required for the tests. They include serum samples from the normal inhabitants of different regions in Colombia (Pacific coast, Magdalena river valley, San Vicente de Chucurí area, etc.). Two sera were obtained from wild animals.

Virus. The Kubes-Rios strain of the VEE virus, kindly supplied by Dr. Max Theiler of The Rockefeller Foundation Virus Laboratories in New York City, was used both for the preparation of the hemagglutinating antigen and for the N tests. The virus itself was maintained in ampoules stored in CO_2 ice as a 10% suspension of infected infant mice brains in 0.75% bovalbumin prepared in buffered saline.

Hemagglutination-inhibition test. The antigen was prepared and preserved according to methods described elsewhere.[1] The technique employed was essentially that of Clarke and Casals.[4] In the first 182 specimens bentonite was used to remove the nonspecific inhibitors. For all the other sera, kaolin was utilized for the same purpose; in the case of animal sera, acetone extraction was also carried on as a control method.[10]

Neutralization Tests. Equal volumes of serum and properly diluted VEE virus were mixed and incubated for 2 hours at 37°C. Each mixture was injected intraperitoneally into six adult, albino-Swiss Rockefeller mice, with an inoculum of 0.1 ml to each animal. The test animals were observed for 12 days. Between 50 and 150 LD_{50} were employed in all tests according to the method of Reed and Muench.[9] As a diluent, 0.75% Armour's fraction V of bovine plasma was routinely used. Survival ratios of 6/6, 5/6, 5/5, and 4/4 were considered as positive; those specimens with ratios of 0/6, 1/6, 2/6, 0/5, 1/5, and 0/4 were regarded as negative.

RESULTS

Table 1 summarizes the results obtained in the comparative study here described.

DISCUSSION

The analysis of Table 1 shows two well-defined facts: 1) in all 344 specimens in which no HI antibodies could be demonstrated there was also a negative result in the N test; 2) in 100 sera with HI titers of 1:80, 1:160, 1:320 or higher, the N test was always positive.

Among the 43 sera exhibiting HI antibodies in titers of <1:10, 1:10, 1:20 and 1:40, there are 30 instances in which the N test was positive and 13 in which it was negative. Table 2 includes these 13 sera and the results obtained when as-

TABLE 1

Comparative results of tests for antibodies to VEE virus in sera studied by the HI and the N tests

HI test		N test	
Sera tested	Titer*	Neg.	Pos.
344	0	344	0
2	<10†	1	1
10	10	6	4
10	20	1	9
21	40	5	16
26	80	0	26
34	160	0	34
40	320+	0	40

* Expressed as the reciprocal of the highest serum dilution inhibiting hemagglutination.

† In all tests the first dilution of serum was 1:10. In these cases the first tube showed only partial inhibition.

sayed by the hemagglutination test with other members of Casals' group A of viruses.[1] Unfortunately, due to the small volumes of sera available, it was impossible to include every one of them in all these comparative tests.

It would appear, after examining this table, that in nine out of the ten sera that were tested for antibodies to Mayaro virus, this particular virus, or a closely related agent such as Semliki or Chikungunya, could be incriminated as the cause of the HI antibodies demonstrated in the test with the VEE antigen, due to the immunological cross reactions within the members of the group A of arthropod-borne viruses.[2]

Case No. 7 could represent either a nonspecific reaction with the VEE antigen or a serological overlap due, perhaps, to a hitherto unknown virus belonging to group A.

It is worth mentioning that all sera giving any titer in the HI test with the Eastern equine encephalitis virus (EEE), were found to be negative in the N test with this same virus.

After these considerations and with the results herein reported it is reasonable to assume that for screening purposes the VEE HI test is the method of choice, leaving for the N test only those sera showing relatively small amounts (titers less than 1:80) of HI properties.

Consequently, with this test we are inclined to believe that for this type of epidemiological survey work all those sera inhibiting hemagglutination in titers of 1:80, or higher, could be considered as true positives, in which case the N test should be limited to samples with titers in the in vitro reaction of <1:10, 1:10, 1:20 and 1:40.

TABLE 2

HI tests for Group A arthropod-borne viruses in sera showing HI but no N antibodies to the VEE virus†

Case No.	VEE	WEE	EEE	Mayaro	Semliki*	Chikungunya*	Sindbis*
1	<10	10					
2	10	20	20	1280			
3	10	20	20	320+			
4	10	20	20	320+			
5	10	20	80	320+			
6	10						
7	10	0	0	0	0‡	0‡	0‡
8	20	0	80	320+	0‡	40	20
9	40	0	20	320+			
10	40	20	10	640			
11	40	0	40	320+			
12	40	80	160	320+			
13	40	80					

* Test done by Dr. J. Casals at the Rockefeller Foundation Virus Laboratories, New York.

† The figures indicate the reciprocal of the highest dilution of serum inhibiting hemagglutination.

‡ First dilution of serum tested was 1.20.

SUMMARY

1. Studies were made of 487 sera for antibodies to the VEE virus both by the hemagglutination-inhibition (HI) and the neutralization (N) tests.

2. Of 344 sera that were negative in the HI test all were negative in the N test. Of 100 sera that exhibited titers of 1:80 or higher in the HI test all neutralized the virus. Among 43 sera with HI titers of <1:10 to 1:40 there were 13 that failed to neutralize the virus. Tests on these 13 sera indicated that another group A virus could have been responsible for the HI results, and in nine instances it was established that the Mayaro or another virus closely related to it might have been responsible for the production of the VEE HI antibodies of low titer.

ACKNOWLEDGMENTS

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THE TREATMENT OF PINWORM INFECTIONS IN HUMANS (ENTEROBIASIS) WITH PYRVINIUM CHLORIDE AND PYRVINIUM PAMOATE

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INTRODUCTION

Pinworm infections still constitute an important medical problem to the pediatrician as evidenced by a cursory survey of the literature. Many drugs have been tested for their efficacy against *Enterobius vermicularis* with varying results.

Previous work[5] has indicated the activity of a cyanine dye #715 against pinworms in laboratory animals. This drug, later designated as pyrvinium chloride, was shown[3, 4] to have a cure rate approximating 100% for pinworms in humans. Recently,[1] a comparative study of two pinworm drugs in humans also showed a 100% cure rate for pyrvinium chloride.

The study herein reported was carried out to test further the efficacy of this drug. During the study an improved pamoate form of the drug became available and this was then included in the investigation. Summarized results of some tests in mice and rats on oral toxicity of the two drugs[2] are also included for comparative purposes.

MATERIALS AND METHODS

Two institutions, housing children of all ages, were employed in the program. The smaller home, St. Joseph's Villa, Miami, Florida, contained approximately 15 to 20 children at any given time. Sexes were mixed but all children were white. The larger institution, the Dade County Home at Kendall, Florida, also contained children of mixed sexes, but Negroes as well as whites were present. The total number in this institution at the beginning of the study averaged around 175. Infected families, chosen more or less at random from among the medical school faculty and student body, were also employed.

All children used in the multiple-dose study were Scotch-taped every morning before rising for 7 consecutive days. On this basis positives and negatives were obtained. Scotch-taping was continued thereafter on both treated and untreated patients during the treatment-time period of 7 days, and for the next 30 consecutive days. Thus, Scotch-taping was attempted on all patients, as far as possible, for a total of 44 consecutive days.

Negative controls consisted of two groups, one untreated and the other treated but both Scotch-taped for the 44-day period. Positive controls consisted of a series of untreated positives, likewise Scotch-taped for the 44-day period.

Positive cases receiving the single dose therapy were Scotch-taped for 14 consecutive days following treatment.

A careful check was kept on all patients during and following the treatment period for any reactions to the drug.

The drug employed in both the chloride and pamoate form, in the multiple dose study, was given orally at the dosage rate of 2 mg/kg body weight divided into 3 doses daily for 7 consecutive days. Both forms of the drug were supplied in a raspberry-flavored syrup.

In the single dose study only the pamoate drug was used and, likewise, was given orally, but at the dosage rate of 5 mg/kg body weight. The drug was administered predominantly in the syrup form but, in a few instances, capsules were employed.

TESTS IN ANIMALS[2]

Acute Oral Toxicity in Mice. Animals given pyrvinium chloride at the 4 and 6 mg/kg dose level showed slight depression in 1 hour but a return to normal thereafter. Doses of 8 and 10 mg/kg produced similar reactions but one animal receiving the 8-mg dose died in 18 hours and 2 receiving the 10-mg dose died in 18 hours and 4 days, respectively, following administration.

Animals given pyrvinium pamoate at the dosage rate of 50 to 125 mg/kg showed slight depression from 30 minutes to 1 hour after treatment but subsequently returned to normal. No further reactions were observed in animals given up to 300 mg/kg.

Oral Tolerance in Rats. Animals receiving 14 mg/kg of pyrvinium chloride daily for a period of 1 to 4 weeks showed moderate food-intake depres-

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TABLE 1

Results of Scotch-taping over 37-day period on treated and untreated positive and negative cases

Drug used 2 mg/kg divided tid.	No. of cases	Treatment period (7 days)	Post-treatment period (days)			
			1–7	8–14	15–21	22–30
			Cure rate among treated positives			
Pyrvinium chloride	63 (57W)* (6N)*	57 (90.4%) (53W—92.9%) (4N—66.6%)	61 (96.8%) (55W—96.4%) (6N—100%)	63 (100%)	63 (100%)	63 (100%)
Pyrvinium pamoate	34W	32 (94.1%)	34 (100%)	34 (100%)	34 (100%)	34 (100%)
			Spontaneous cure rate—untreated positive controls			
Untreated	14W	4 (28.5%)	4 (28.5%)	4 (28.5%)	4 (28.5%)	4 (28.5%)
			Negative controls becoming positive			
Untreated	86 (37W) (49N)	1 (1.1%)	2 (2.3%)	5 (5.8%)	5 (5.8%)	7 (8.1%) (6W—18.9%) (1N—2.0%)
Pyrvinium chloride	33 (17W) (16N)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

* W = white; N = Negroes.

sion and severe weight-gain depression, while those given 31 mg/kg showed similar reactions with very severe weight-gain depression but no significant blood changes.

Animals receiving 198 mg/kg of pyrvinium pamoate daily for a period of from 2 to 4 weeks showed a marked food-intake depression with an accompanying severe weight-gain depression but no significant blood changes.

RESULTS OF THERAPEUTIC TRIAL

PYRVINIUM CHLORIDE STUDY. In the study employing pyrvinium chloride a total of 196 patients (176 from the County Home; 18 from the Villa) were tested for the presence of pinworms. Of this number 77 (39.2%) were infected. Of the 196 tested 71 were Negroes and 125 were whites. The highest incidence of infection, as would be expected, occurred in the white population with 71 (56.8%) being positive as opposed to 6 (8.4%) among the colored. Infections occurred predominantly in the 6–12-year age group with equal distribution among the sexes.

As shown in Table 1, 63 positive cases were treated with pyrvinium chloride at the dosage previously indicated. 57 (90.4%) became negative during the treatment period and remained negative throughout the course of the study. An additional four, total 61, (96.8%) became negative within the week following treatment and all were negative by the 9th day (2nd day of the 2nd week) following treatment and remained negative throughout the study. The presence of positive slides in one case on the 24th and 27th day following termination of treatment can be explained as a technical error or a very early reinfection, since pinworm ova were absent for 23 consecutive days prior to this time. Of 14 positive cases left untreated, 4 (28.5%) became negative spontaneously during the first week or in the treatment-time period following determination of infections. They remained negative during the course of the study. Of 86 untreated negative controls 7 (8.1%) became positive before termination of the study. Further breakdown between whites and Negroes is given in the Table. In all, 33 negative controls, 17 of whom were white and 16 colored, were treated with pyrvinium chloride. None became positive at any time during the course of the study. During the entire study occasional days of Scotch-taping were missed in various patients, but not with sufficient frequency to interfere with an accurate interpretation of results. Data obtained on all cure rates were found to be statistically significant at the 1% level.

Four months following this study St. Joseph's

Villa was rechecked for reinfection. At this time, 15 children were present, 14 of whom had been present in the previous study. With the exception of one child who was negative as before, all 13 were again positive for pinworms. One new admittance was also positive.

PYRVINIUM PAMOATE STUDY. (A) MULTIPLE DOSE SCHEDULE. Ten months following the pyrvinium chloride study the pamoate form of this cyanine dye became available. A study was carried out at both institutions employing 19 positive patients from the Villa and 15 from the County Home. As indicated in Table 1, 32 (94.1%) became negative during treatment, 26 (81.2%) being negative after 4 days of therapy. All were cured within the first week following termination of treatment, remaining negative throughout the study. As in the case of the chloride study some days of Scotch-taping were missed but were not sufficient to interfere with an accurate interpretation of results. One child passed an *Ascaris lumbricoides* adult during the treatment period.

(B) SINGLE DOSE SCHEDULE. Because of the good results achieved with the multiple dose schedule it was decided to investigate the curative value of a larger but single dose of this drug. One hundred positive patients consisting of inmates of the institutions mentioned, as well as the families of some medical school faculty and students, were treated with a single dose (5 mg/kg) of pyrvinium pamoate. Scotch-taping was continued for 14 consecutive days following treatment. As indicated in Table 2, the day following treatment 45 (45%) were negative by Scotch-taping and remained negative throughout the 14-day period of testing. This number increased to 62 (62%) on the 2nd day and so on, until a cure rate of 96 (96%) was reached on the 7th day. Of the four patients showing positive slides during the 2nd week, two in each case showed only one egg per slide but once during this week.

TABLE 2

Results of Scotch-taping over 14-day period following treatment of pinworm patients with a single dose of pyrvinium pamoate (5 mg/kg)

No. of cases	Per cent cure rate (*days*)							
	1	2	3	4	5	6	7	8–14
100	45	62	77	88	94	95	96	96

REACTIONS TO TREATMENT. Pyrvinium chloride was not well received by the children. Complaints of a bitter taste were common and nausea and vomiting occurred on several occasions.

Pyrvinium pamoate, on the other hand, was well received and no complaints made by any of the patients involved. Many of those who had the chloride form previously remarked about the contrast in taste and expressed a liking for the raspberry flavored pamoate drug.

DISCUSSION

From the data presented it is apparent that both pyrvinium chloride and pyrvinium pamoate are comparable in curative value, both effecting a cure rate of over 90% during the treatment time period of 7 days and a 100% cure within the 2-week period following treatment. The marked difference in toxicity in animals, however, shows the pamoate (approximately ¼ as toxic) to be superior to the chloride form.

The curative value of a single dose of pyrvinium pamoate (96%) represents a marked advance in pinworm therapy. Heretofore most pinworm treatments have consisted of therapy several times a day for several days or even weeks. The popularity of the single-dose regimen over the multiple-dose schedule was quite noticeable among the mothers of the children being tested.

In view of the prevalence of pinworm infections in institutions as well as in the average family, and the ineffectiveness of personal hygiene and public sanitation in preventing reinfection, the establishment of periodic "wormings" might well be considered. With the advent of a single-dose regimen this seems quite feasible, and the removal of pinworms as a personal and public health problem might well be in sight.

SUMMARY

A pinworm study employing pyrvinium chloride and pyrvinium pamoate is described. Differences in toxicity indicate the superiority of the pamoate over the chloride form, though both are equally efficacious in therapeutic value. The pamoate drug was shown to be more pleasant to the taste and caused no gastrointestinal disturbances.

Employment of 2 mg/kg body weight divided tid for 7 consecutive days resulted in a 100% cure rate for both forms of the drug.

Pyrvinium pamoate given in a single dose of 5 mg/kg effected a cure rate of 96%.

All cure-rate data obtained were found to be statistically significant at the 1% level.

The value of periodic "wormings" is discussed and its feasibility with the use of pyrvinium pamoate in a single dose regimen is indicated.

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THE DIARRHEA OF TRAVELERS

II. Bacteriologic Studies of U. S. Students in Mexico*

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The diarrhea of travelers, widely recognized as a clinical syndrome, has been the subject of more speculation than investigation. During the past decade many informative studies as to the etiology of diarrhea in infants and adults at home and abroad have been reported, but the relationship of these investigations to the diarrhea of travelers remains obscure.[1, 2, 3] Clinical aspects of the diarrhea of travelers, evidence that it is not parasitologic in character, its incidence in travelers to Europe and Mexico and other epidemiologic data have already been reported.[4, 5]

This study was designed to determine if any of the enteropathogenic bacteria were responsible for the clinical syndrome. In the past, investigations were conducted on individuals *after* they became sick. Since some bacteria may be cultured only briefly after the onset of symptoms, and since there has been increased interest in the changing intestinal flora following illness, it was decided to culture stools of patients *before*, *during*, and *after* their attacks of diarrhea.

Mexico City was chosen as the site for these investigations because previous work had indicated that at least 35% of tourists became ill within a fortnight of arrival[5] and because of the bacteriologic facilities available.

Methods

A total of 74 U. S. students recently arrived in Mexico were asked to provide a stool specimen for 14 consecutive days; 62 cooperated.

Of the 62 students completing the 14-day period, 23 were males and 39 were females. The average age of the males was 23.6 years; of the females, 21.1 years. The students had been in Mexico from 1 to 11 days before providing their first specimen; the average was 3 days, the median being 2 to 3 days. All were given a placebo twice daily as part of another experiment and to insure their avoidance of other drugs.

The students were requested to bring in each day a piece of toilet paper which was heavily stained or lightly embedded with feces. The paper was submitted in an envelope which contained the date, the code number of the student and an additional card which indicated whether or not the student had diarrhea on that day. The card was removed before the specimen reached the laboratory so that the bacteriologic studies were done with no knowledge of which students were ill. After the 14-day routine study, the students were requested to bring in samples for an additional fortnight if they became ill.

Those students who contracted diarrhea were visited at their residences or interviewed immediately after recovery and a history was obtained.

Students were classified as to severity of illness in the following manner:

1) *Mild:* those with three or more bowel movements a day for 1 or more days, or with two watery stools, or nausea or cramps
2) *Moderate:* those with nausea, cramps, vomiting or fever accompanying diarrhea of 1 to 2 days' duration
3) *Severe:* those with nausea, cramps, vomiting, fever, and diarrhea of at least 2 days' duration, including confinement to quarters for 1 day.

Bacteriologic Techniques

The specimens were handled by the tissue or toilet-paper technique described by Lie Kian Joe.[6] Most of the specimens reached the laboratory within 24 hours but all were examined within 3 days. (Examination of several thousand specimens submitted to our laboratory from several parts of Mexico had established fully the value of this technique for the isolation of enteropathogens including *Shigella* and *Salmonella*.[7, 8] Similar results have been reported from other parts of the world.[6])

The following organisms, *for purposes of classi-*

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§ Medical Student of the National Foundation.

TABLE 1

PATHOGENS OF SICK STUDENTS

CASE NO.	I	1	2	3	4	5	6	7	8	9	10	11	12	13	14	ILLNESS
2	2		EC 026			■		EC 0111							■	MODERATE
12	3								PARA		KLEB	■	■	■		SEVERE
14	3					EC 086						■	■	■		SEVERE
16	2	EC 044									PARA					MILD
18	2					■			PARA							MILD
19	2							■								MILD
24	3										■					MILD
28	3		■	■			EC 0128									SEVERE
29	2								PARA	PARA		PARA			PARA	SEVERE
32	2									PARA	■					MILD
33	2						■	■	■		PARA	KLEB		PARA	PARA	MILD
35	7										KLEB		PARA		■	SEVERE
36	3						EC 0128			KLEB				■		MILD
39	2					■										MILD
44	1						EC 0111 PARA	■								SEVERE
49	1					■		■		■				■		MILD
50	1				■	LEC DIFF										SEVERE
51	1				EC 0111 KLEB	■	■	■	PARA			■	■			MILD
52	3				■	■	■	■	■	PARA	KLEB	■	KLEB	PARA		MILD
55	9			■	■							PROTEUS PARA				MODERATE
57	4					■		PARA			KLEB		PARA			MILD
61	3	■	PARA				■	■								MILD
69	1						■									MILD
70	4								PARA	■	■	■				MILD
71	2						O	O								SEVERE
72	4		■	■	■	■		KLEB	KLEB							SEVERE
73	3			■	■	■	■	■	■	■	■	■	■	PARA		MILD

Column headed by Roman numeral I indicates number of days after arrival in Mexico before participation in study.

Days in black indicate days of diarrhea.

Symbol O in Case #71 means no specimen submitted on that day.

fication only, were considered pathogenic or possibly pathogenic:

1) *Salmonella* sp.
2) *Shigella* sp.
3) *Klebsiella* sp.
4) *Paracolobactrum* sp.
5) *Proteus* sp.
6) *Escherichia coli* 026, 044, 055, 086, 0111, 0112, 0119, 0124, 0125, 0126, 0127, 0128.

All other organisms were considered nonpathogenic. Special cultures for *Staphylococcus* sp. were not made.

Details of Bacteriologic Technique

The stained portion of the toilet paper was cut out and incubated in a test tube of infusion broth for 1 hour at 37° C. It was then inoculated on Endo and SS agar, and incubated for 24 hours at 37° C.

The colonies which grew out were separated into *lactose positive* and *lactose negative*.

Lactose Positive. Lactose-positive colonies were cultured on infusion agar. Twelve colonies of each plate were then tested with three separate polyvalent serums of *E. coli* pathogens. The serums were as follows:

a) contained 026, 044, 055, and 086;
b) contained 0111, 0112, 0119, and 0124;
c) contained 0125, 0126, 0127, and 0128.

Any colony that agglutinated with one of the polyvalent serums was then tested in a second slide agglutination with each member of the group separately. This test isolated the suspected O antigen. To destroy other or overlapping antigens (B, L, etc.) that might give a false positive test, the culture of the positive colony was then boiled for one hour (93° C in Mexico City). A third slide agglutination was then performed.

TABLE 2

Pathogens of well students

Case No.	I	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3	5												Kleb	Para	
6	9							Para							
7	9														
8	3														
9	5								Para	Para					
10	9														
11	3								Para						
13	3														
15	2														
17	2		Kleb							Para					
20	1														Kleb
21	2														
22	2											Para			
23	2							Eco126							
25	2		Kleb							Para			Para		
26	3														
27	2														
30	2														
31	2										Para		Para		
34	11														
38	11														
40	2														
42	2														
43	2														
45	2							Para							
46	1							Para							
48	1		Eco26												
53	1														
56	3					Kleb									
58	3								Para		Para				
60	3														
62	2														
63	2														
67	4							Para							
69	2														

Column headed by Roman numeral I indicates number of days after arrival in Mexico before participation in study.

When this appeared positive a final check was made by using serial dilutions in test tubes incubated for 18 hours at 50° C. To be considered positive, the agglutination had to be the same as the specific titer code for each *E. coli* pathogen being tested.

E. coli pathogens found by the above method were recultured on fresh medium and sent to Paris for determination of the lyso-type.

Klebsiella sp. was identified by appearance and biochemical reactions (lactose positive, indole negative, nonmotile and with a capsule).

Lactose Negative. Lactose-negative colonies were cultured on Kligler agar, mannitol and Surraco broth (urea and lactose), and Sim media, for 24 hours at 37° C. All lactose-negative colonies were tested with a polyvalent serum if the findings were suggestive of *Salmonella*. The polyvalent serum contained all the somatic antigens of all groups of *Salmonella*. If the biochemical results were suggestive of *Salmonella*,

TABLE 3

Summary of all organisms cultured during 14-day period

	Well (35)	Sick (27)
Only nonpathogens, including enterococcus and nonpathogenic *E. coli*	19	6
Paracolobactrum sp.	12	15
Klebsiella sp.	5	8
Pathogenic *E. coli*	2	9
Proteus mirabilis	0	1
Shigella sp.	0	0
Salmonella sp.	0	0

but the colony *failed* to agglutinate with the polyvalent serum, the colony was tested with a polyvalent serum of each of the separate groups of *Salmonella*.

Shigella sp. was identified by biochemical reactions only.

All lactose-negative colonies that did not fit into any group biochemically were designated as *Paracolobactrum*.

Proteus were identified by biochemical means only, differentiation being made among *P. vulgaris*, *P. mirabilis*, *P. morgani*, and *P. rettgeri*.

Candida was identified on the basis of morphology only.

Results

These are recorded in detail in Tables 1 and 2 and summarized in Table 3. Of the females 16 (41%) and of the males 11 (48%) contracted diarrhea during the 14-day period.

During the illness of the 27 sick students 18 had no pathogens in their stools, five had *Paracolobactrum*, one had *Paracolobactrum* and *Klebsiella* sp., one had *E. coli* 0128, one had *E. coli* 0126 and *Paracolobactrum* and one had *E. coli* 0126 and *Klebsiella* sp. If the period of illness is broadened to include 2 days before the illness, and two days afterwards, then of the 27 sick students 11 had no pathogens, nine had *Paracolobactrum*, two had *Klebsiella* sp., one had *E. coli* 0111, one had *E. coli* 0128, one had *E. coli* 0126 and *Paracolobactrum*, one had *E. coli* 0126 and *Klebsiella* and *Paracolobactrum* and one had *Klebsiella* and *Paracolobactrum*.

Role of Enteropathogenic E. Coli. The enteropathogenic subgroups of *E. coli* may be considered in more detail. *E. coli* 0126 was cultured in three sick students: one 3 days after illness (severe), two on the day of onset (one severe and the other mild). *E. coli* 0126 and *E. coli* 0111 both were cultured in the stool of one student with moderate diarrhea, the former 3 days before illness and the latter 2 days after the illness. *E. coli* 055 was cultured in one student 6 days before severe illness. *E. coli* 044 was cultured in one student 9 days before moderate illness. *E. coli* 0125 was cultured in one student 6 days before mild illness. *E. coli* 0128 was cultured in one student on the second day of severe illness. During the 14-day period only two well students had enteropathogenic *E. coli*; these were *E. coli* 026 and *E. coli* 0126.

In no student, whether sick or well, were enteropathogenic *E. coli* cultured for more than 1 day. It was in pure culture on the day of its appearance except in two cases in which *E. coli* 0128, appeared once with *Paracolobactrum*, and once with *Klebsiella*.

Diarrhea After 14-Day Study. Diarrhea occurred in a total of nine students following the initial 14-day study. Of these, four had been well during the primary 14-day period, two developed a diarrhea toward the end of the primary period that continued into the second 14-day period of observation and three had a recurrence of diarrhea. Of the four who had been well only one had had a pathogen (*Paracolobactrum*) cultured during the primary 14-day period. Of the three listed as sick and who developed a recurrence one had had *E. coli* 044 and *Paracolobactrum* cultured during the initial 14-day period, one had had *Paracolobactrum* and one had had no pathogen cultured. Cultures of these seven during the period of late diarrhea (15 to 28 days after beginning of initial study) revealed *Paracolobactrum* in one and *Proteus morgani* in one.

In six other students who had been listed as sick during the primary 14-day period cultures were taken at random from the 19th to the 25th days. *Paracolobactrum* was found in one and *Proteus morgani* in another.

Discussion

Of the 27 students who contracted diarrhea, six had no bacterial pathogens at any time during the 2-week period of study, whereas of the 35 well students 19 had no pathogens. However, during the period of diarrhea 18 or two-thirds had no pathogens in the stool whereas in the control

group pathogens were cultured in 16 of the 35 at least once during the 2-week period. There was a higher incidence of *E. coli* enteropathogens among those who contracted diarrhea than among those who did not. The difference in appearance of *Klebsiella* sp. and *Paracolobactrum* as between those who were well and those who were sick was not great enough to warrant any conclusion as to their relationship to diarrhea, although it is possible that in individual cases they may have been the responsible pathogen. It may be significant, however, that of the nine severe cases, eight had *E. coli* enteropathogens, *Klebsiella* sp. or *Paracolobactrum* sp. at one time in their stools.

The absence of *Salmonella* and *Shigella* organisms, which had been thought to be among the major causes of tourists' diarrhea, is noteworthy.

Although the bacteria considered by definition to be pathogenic were found more often in those with diarrhea than in those who remained well, a definite causal relationship was not established. It is possible that bacterial enteropathogens may appear in greater numbers and variety in individuals who develop diarrhea from other causes, *e.g.*, viruses or *Staphylococcus* toxin.

Summary

Stool samples were obtained for 14 consecutive days from 62 United States students in Mexico.

Of these 27 became ill with diarrhea and 35 remained well during the period of observation.

Salmonella and *Shigella* were not cultured in any case.

Enteropathogenic *E. coli*, *Klebsiella* and *Paracolobactrum* could not be incriminated as the cause of the diarrhea of travelers to Mexico.

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RELAPSE OF LEPROSY IN AMERICAN SAMOA

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One usually associates endemic leprosy in the United States and its possessions with the Gulf States and the Hawaiian Islands. Rather less attention has been directed to American Samoa, though the incidence of the disease is far higher than elsewhere on United States soil. In 1953, Gray brought attention to American Samoa as an important endemic focus of leprosy. Since then the known incidence has more than doubled.

During a 6-months period in 1957 and 1958, the author conducted a general survey of leprosy in American Samoa. The results of the survey provide an epidemiological description of leprosy in a small and somewhat isolated population. In addition, it is felt that the data disclose features of the natural history of sulfone-treated leprosy which have bearing on clinical management and public health policy wherever the disease occurs. In particular, the author wishes to emphasize the tendency of lepromatous leprosy to undergo reactivation in spite of protracted chemotherapy with sulfone, and the tendency, on the contrary, of tuberculoid leprosy to undergo spontaneous cessation of activity even in the face of inadequate chemotherapy.

THE SAMOAN ISLANDS AND PEOPLE

The Samoan Islands are a volcanic group in the tropical South Pacific populated by Polynesian people of relatively unaltered culture and race. Administratively, the islands are divided into a New Zealand trust territory and the United States possession, the former including the bulk of the land and the majority of the people. American Samoa has a population of 20,154, of whom 86% live on the island of Tutuila.

Nine-tenths of the Samoan population is racially unmixed, and there are less than 175 persons of exclusively European extraction in American Samoa. Native tradition is still very strong; the clan and the village are the basic social units, and most local authority is invested in clan chiefs. Families live in one-room, open thatched houses. The majority of villages are confined by the rugged terrain to the narrow strip of flat land between the beach and volcanic peaks. The economy is based on subsistence farming of the traditional Pacific staples, supplemented by cash earnings from the sale of handicraft and employment by the territorial government and a local fish cannery. Until the last decade Samoan society was able to reject or assimilate Western influences without compromising their traditional way of life, but this has become increasingly difficult at the present time.

The robust and massive physique of the Samoan suggests vigorous health to the visitor, but health problems are numerous. Filariasis and trachoma are hyperendemic, and the primitive sanitation results in almost universal intestinal parasitism. Pulmonary disease, rheumatic heart disease, sepsis, and infantile malnutrition contribute to a high mortality rate.

LEPROSY IN AMERICAN SAMOA BEFORE 1957

Introduction of Leprosy to Samoa. Samoan folklore ascribes affliction with leprosy to a curse on the victim by a ghost ("aitu"), usually a dead ancestor. The most reasonable theory of introduction of leprosy to Samoa is that it was brought by the Chinese coolies who were imported to Western Samoa in the 19th century as plantation workers. Gray presented evidence in 1953 that most of the cases in American Samoa had sprung from two leprous Samoans who had immigrated from Western Samoa in the second decade of this century. Certainly there is little to suggest that leprosy has been present in Samoa since antiquity.

Detection. The first diagnosis of leprosy in American Samoa was made in 1917, 17 years after the United States was ceded possession of the territory. Until 1943, only ten cases had been diagnosed, but in 1944 detection rates climbed to an average of seven new cases a year for the next 13 years. Of the new cases detected in American Samoan persons 17 were diagnosed outside of American Samoa. It is probable that this increase

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in detection was due to an increased true incidence rather than simply to an increased medical awareness of the disease.

In 1953 an epidemiological leprosy survey was made in American Samoa under the auspices of the South Pacific Commission. Four thousand persons were examined and six new cases of leprosy were detected. The incidence of the disease was calculated to be 2.4 per thousand at that time.

Facilities for Care. Until 1953 American Samoa had no leprosarium. Occasional leprosy patients were treated in the general hospital in Tutuila, American Samoa, but the majority were sent to Makogai, the Crown Colony of Fiji, where they remained until the disease was arrested. Between 1950 and 1953 most of the American Samoans at Makogai received sulphetrone, 3.0 grams daily. In 1953, through the efforts of a former governor of American Samoa, a 30-bed leprosarium was constructed in Tutuila, making it possible for the American Samoans at Makogai to be repatriated.

The following program was in effect from 1953 until September, 1957. Bacteriologically-closed cases were treated as outpatients; open cases were confined to the leprosarium. All new cases underwent skin biopsy, and the histological diagnosis was made by the Armed Forces Institute of Pathology. Skin scrapings were performed every 3 months on inpatients. When no acid-fast bacilli were seen on three such successive scrapings, the patient's disease was considered to be arrested and he was released to outpatient care. There was no effective outpatient follow-up program. Treatment of inpatients and outpatients was with the drug, sulfoxone sodium, two tablets of 0.33 gram six times a week with a rest period 1 week in 4, or with diaminodiphenyl sulfone (DDS), 0.3 gram biweekly, with a lower dosage initially.

THE GENERAL LEPROSY SURVEY OF SEPTEMBER, 1957

Methods. Records of all old cases were assembled and summarized. Each old patient was brought to the clinic where he was given a physical examination for evidence of leprosy and where skin scrapings for acid-fast bacilli were done from multiple sites. An endeavor was made to obtain a full-thickness skin biopsy from each of the tuberculoid cases. Biopsies were sectioned and interpreted by the Armed Forces Institute of Pathology. All patients had a blood count, urinalysis, and photofluorographic chest film. Each patient was started on the follow-up schedule which was devised in the Colony of Fiji: quarterly visits for 3 years, biannual visits for 3 years, and annual visits thereafter. Treatment was reinstituted in nearly all cases, and the dose of DDS was increased to 400 milligrams biweekly. Old patients whose skin scrapings showed acid-fast bacilli were reconfined to the leprosarium, and disease activity was confirmed by skin biopsy.

Results of the Survey. In all, 58 old leprosy outpatients were located and examined by the author personally. Twenty other old patients were discovered to have emigrated from American Samoa, and one old patient could not be located. During the 6 months of the survey, the public health nurses in the villages were alerted to leprosy by letter and lecture; ten new active cases were detected (5 lepromatous and 5 tuberculoid), seven by public health nurses. Of the 58 old outpatients who were examined, seven with lepromatous leprosy were found to have acid-fast bacilli in skin scrapings and were reconfined to the leprosarium.

At the conclusion of the survey in March, 1958, 101 living persons with active or arrested leprosy were accounted for. Subsequently it was determined that an additional six American Samoans had been treated at the U. S. Public Health Service Hospital in Carville, Louisiana, making the incidence of known leprosy 5.3 per thousand. American Samoan leprosy patients who had left Samoa were included when calculating the incidence, as it was felt that their disease was of Samoan derivation. In fact, 12 were known to have had leprosy at the time they departed from Samoa; the 14 others were diagnosed elsewhere after they had left Samoa. Immigration laws which prohibit entry to the United States of persons afflicted with leprosy do not apply to American Samoans, as they have the status of U. S. nationals.

The distribution of known leprosy in American Samoa is given in Table 1.

Geographical Distribution. In American Samoa leprosy is not a disease of the remote village. Almost half of the leprosy patients come from five large villages on Pago Pago Bay. In the village of Pago Pago the incidence is 10.7 per thousand. The higher incidence of the disease in the more urbanized areas might be ascribed to better de-

TABLE 1

Distribution of leprosy cases in American Samoa

March, 1958

I. Patients currently in residence in American Samoa	81
A. Lepromatous type	40
1. Inpatients, bacteriologically positive	24
a. Initial hospitalization	15
b. Re-entries	9
2. Outpatients, bacteriologically negative	16
B. Tuberculoid type	34
C. Indeterminate type	7
II. Patients who have left Samoa	26
A. Diagnosed in American Samoa	12
B. Diagnosed in Hawaii	7
C. Diagnosed in U. S. A.	7
III. Total known cases	107
IV. Missing	1
V. Dead	15

TABLE 2

Rates

Case-type rate: number open cases per 100	29.6
Sex rate: number male patients per 100	65.4
Childhood rate: persons aet 0–14 per 100	8.6
Contact rates:	
Number with familial contact per 100	43.2
Number with extrafamilial contact	1.2
Number with contact unknown	55.6
Tuberculosis rate, previously clinically active	7.4

tection, but this presupposes a larger number of undiagnosed cases in remote areas than seems reasonable. The observed distribution is consistent with the view that leprosy was introduced to American Samoa relatively recently.

Rates. The figures in Table 2 were derived from data on the 81 leprosy patients currently in residence in American Samoa, all of whom had been personally examined by the author. Familial contacts consisted of parents or siblings except in two instances where the spouse was the familial contact.

Age Distribution. Due to rapid population growth, there is an extraordinarily high proportion of children in American Samoa: almost half of the population is less than 15 years of age, and slightly under one third are between 15 and 34.

TABLE 3

Percentage distribution of age of leprosy patients

Age group	General population	Leprosy patients	Age when diagnosed
1–4	18.5	0	0
5–9	16.0	1.2	2.4
10–14	13.9	7.4	21.0
15–19	10.7	18.5	19.8
20–24	8.1	13.6	16.0
25–29	6.7	14.8	18.5
30–34	5.9	17.3	3.7
35–39	4.7	6.2	2.4
40–44	3.7	3.7	4.9
45–49	3.2	4.9	3.7
50–54	2.3	4.9	6.2
55–59	2.2	3.7	0
60–over	4.1	3.7	1.2

In contrast, as shown in Table 3, less than a tenth of the leprosy patients were under 15, and two-thirds were between 15 and 34. Of the leprosy patients, 22.4% were under 15 when their disease was first detected. The median age at time of diagnosis was 22.

Lepromatous Leprosy. The most striking finding of the survey was that, of 22 old lepromatous outpatients examined, seven had acid-fast bacilli in scrapings of their skin. In addition, records disclosed that two of the active lepromatous cases which were in the leprosarium at the start of the survey were old patients who had once been considered arrested but who had relapsed and had re-entered the leprosarium. Still another patient had relapsed, been reconfined, and had been discharged a second time. Thus, of 24 old patients with lepromatous leprosy who had been released from inpatient care on the presumption that their disease was arrested, ten were, or had been found to be, bacteriologically positive. To confirm the skin scrapings, all ten cases underwent skin biopsy at the time relapse was discovered, and without exception the biopsies showed lepromatous leprosy. In 6 of the 10 cases the histological diagnosis was "active lepromatous leprosy." The remaining four were interpreted to be "recessive," "well resolved," or activity was unspecified. Of the six histologically-active cases, four had conspicuous and extensive macular or nodular infiltration of the skin, and two had less striking signs consisting mainly of peripheral nerve enlargement and swollen ear lobes. Of the

TABLE 4

Percentage distribution of type of chemotherapy used in old lepromatous cases

	Sulphetrone	DDS	Diasone	Other
Bacteriologically positive	20	57	16	7
Bacteriologically negative	33	60	7	0

TABLE 5

Duration and cessation of chemotherapy of old lepromatous cases

	No. cases	Average total months chemoth.	Average months since chemoth. stopped
Histologically active, B+	6	44 (30–66)*	27 (22–37)
Histologically not active, B+	4	47 (30–58)	2 (0–5)
Bacteriologically negative	12	61 (19–93)	6 (0–25)†

* Ranges are given in parentheses.
† Median of 0.

remaining four cases (bacteriologically-positive, but not diagnosed histologically-active), three displayed minimal physical signs consisting mainly of peripheral nerve enlargement, and one was undergoing reaction. It was felt that the six histologically-active cases represented true reactivation of the disease process, and that the four other bacteriologically-positive cases probably represented residual activity rather than reactivation. Reactivation could be due to qualitatively ineffective chemotherapy in the dosage employed, to inadequate total duration of chemotherapy, or to discontinuance of chemotherapy. Residual activity could be due to premature release of the patients from inpatient care. To test these hypotheses, the ten bacteriologically-positive old patients were compared with 12 old lepromatous cases who did not relapse and whose records were adequate. Table 4 shows that the differences between the proportions of positive and negative cases as related to the three sulfones were not great. Furthermore, on pharmacological grounds one would not expect differences in the type of sulfone to be of much significance, as both sulphetrone and Diasone are DDS derivatives and probably are active by virtue of DDS release in vivo. The increased percentage of patients treated with sulphetrone in the bacteriologically-negative group indicates that these patients received slightly more of their treatment at Makogai, Fiji, than the positive group, as this drug was used only at that facility. Dosages were as previously described.

Table 5 shows that lepromatous patients who remained arrested received an average of 15 months more chemotherapy than bacteriologically-positive old patients; but there is considerable overlap between the groups. On the other hand, differences in the time since chemotherapy had been discontinued were striking. The histologically-active group had gone without chemotherapy for an average of over 2 years, and *none had received chemotherapy more recently than 22 months before relapse was detected.* The histologically-not-active but bacteriologically-positive group had gone without chemotherapy for an average of only 2 months; and the bacteriologically-negative group was without chemotherapy for an average of only 6 months and a median of zero months.

One may conclude from these figures the following: 1) The histologically-active group reactivated because their disease was no longer being suppressed by continued chemotherapy. 2) Reactivation occurred in spite of protracted chemotherapy: almost 4 years on the average, and in one case 5.5 years. 3) The bacteriologically-positive group for which there was no evidence of histological activity were probably not reactivated patients, but had harbored lepra bacilli all along and had been released from the leprosarium while still in an open state.

Inpatient care of the bacteriologically-positive, histologically-not-active group was compared with the lepromatous group which did not relapse in Table 6. Though the differences are not great, they do suggest that criteria for discharge from the leprosarium were less firmly adhered to for the bacteriologically-positive group.

In summary, of 24 old lepromatous cases who had been released from the leprosarium, four still harbored lepra bacilli in their skin, and six others were clinically and histologically frankly reactivated as well. Evidence suggests that the former group was probably discharged from the leprosarium while still in an open state, and that the

TABLE 6

Inpatient management of arrested lepromatous cases compared with bacteriologically-positive, histologically-not-active, old lepromatous cases

	Average months inpatient chemoth.	Months between last pos. smear & release	Number negative smears before release
Arrested	38.0	10	2.5
B+, histologically not active	33.5	5.3	2.2

TABLE 7

Distribution of 34 American Samoans with tuberculoid leprosy

I. Old patients	28
A. Biopsied in survey	23
1. No histologic evidence of activity	21
a. Histologic diagnosis of normal skin	10
b. Histologic diagnosis of atrophic skin	5
c. Histologic diagnosis of mild chronic dermatitis	6
2. Histologically active	2
B. Not biopsied in survey	5
II. New patients	6
A. Histologically confirmed active disease	5
B. Without histological confirmation	1

latter underwent relapse because they were not kept on suppressive chemotherapy.

These findings indicate that relapse of lepromatous leprosy may be a more serious problem than it had been considered from the reports of Erickson in 1950 from Carville, and particularly of Lowe in 1954 from Nigeria. Lowe reported a 10.8% relapse rate of old lepromatous cases, and Erickson reported 9% clinical and bacteriological relapse and 9% bacteriological relapse alone.

Tuberculoid Leprosy. As seen in Table 7, of 28 old tuberculoid cases who were examined, skin biopsies were performed on 23. Eighteen of the old patients also had old reports available of the histological confirmation of their initial diagnosis. Of the 23 old patients who were subjected to skin biopsy at the time of survey, there was histological evidence of activity in only two; the rest showed normal skin, atrophic skin, or mild chronic dermatitis. In addition, at the conclusion of the survey there were five new, active, untreated, tuberculoid cases, histologically confirmed. The observation that 91% of the old tuberculoid cases were not active histologically, and that the skin was completely normal in 43% suggested that, unlike lepromatous leprosy, the tuberculoid type tends to become arrested. To see if this difference in the evolution of the two types of leprosy was related to difference in chemotherapy or duration of disease, the data in Table 8 were compiled.

Here it can be seen that the tuberculoid patients received even less total chemotherapy than the lepromatous, and that chemotherapy had been discontinued for an even longer time. From the standpoint of chemotherapy alone, the tuberculoid patients were in a more likely position to

TABLE 8

Contrast in chemotherapy and duration of disease between old tuberculoid and old lepromatous cases

	Patients	Years of disease	Months of chemoth.	Months since chemoth. stopped
Histologically inactive tuberculoid	21	8.0	32	37*
Histologically active tuberculoid	2	2.5	16	20
Old lepromatous, B negative	12	6.3	61	6
Old lepromatous, B positive	10	5.6	45	17

* Range of 0–120 months.

TABLE 9

Frequency distribution of eight physical signs in tuberculoid leprosy

	Histolog. active	Histolog. inactive
Number of patients	7	21
Years since diagnosed	0.4	8
Analgesic macule or plaque	7	1
Enlarged peripheral nerves	2	7
Nerve paralysis	2	6
Cutaneous analgesia	7	12
Muscle atrophy	1	7
Contractures	2	6
Trophic ulcers	1	6
Absorptions of phalanges	0	4

relapse than were the lepromatous patients. The obvious explanation is that it is characteristic of the natural history of tuberculoid leprosy to undergo eventual spontaneous arrest. The general recognition of this is implicit in the inclusion of tuberculoid leprosy in the group designated "benign."

A casual observation was made during the survey that the inactive tuberculoid cases had more striking physical signs than those with histologically active disease. This was investigated in the manner indicated in Table 9 by comparing the frequency of eight physical signs in histologically-active and histologically-inactive tuberculoid leprosy.

The inactive tuberculoid cases commonly showed the conspicuous changes of longstanding denervation: muscle atrophy, contractures, trophic ulcers, and phalangeal absorptions. The group with histologically-active disease showed less trophic changes, but regularly displayed a macule or plaque which was insensitive to pain. The pure neuritic form of leprosy was not encountered. The histologically-inactive patients had had their disease for a much longer time on the average; this probably accounts for their greater affliction with trophic changes.

SUMMARY AND CONCLUSIONS

The incidence of known leprosy in American Samoa was 5.3 per thousand. There were 24 open cases. Of old, outpatient, lepromatous cases, 25% had undergone frank clinical and histological relapse, and 41.7% were bacteriologically positive. Only 9% of old tuberculoid cases showed histological evidence of activity.

Lepromatous leprosy exhibits a strong tendency to relapse when suppressive sulfone chemotherapy is discontinued, even if the patient has previously received chemotherapy for as long as 5 years. Tuberculoid leprosy, on the contrary, tends to undergo arrest of activity, even when chemotherapy has been of brief duration and long since discontinued. Neglect of patient follow-up results in rapid deterioration of leprosy control.

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TWO MALARIA SURVEYS IN THE CENTRAL MOUNTAINS OF NETHERLANDS NEW GUINEA

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INTRODUCTION

The central mountain range of New Guinea stretches south of the equator from about 135° E to 150° E. The part west of the 141st longitudinal is in Netherlands; the eastern part in Australian territory.

In the Dutch part, not much was known about the prevalence of malaria. Two recent surveys, one in the Baliemvalley and one near Homejo in the Kemaboe valley, extended our knowledge somewhat.

Until recently, the inhabitants of both areas lived untouched by Western culture, as late representatives of the stone age. Because clothes are restricted to small aprons for women, while men are practically naked, people look for warmth near fires inside the huts soon after sunset. The staple food is sweet potatoes. Apart from pigs, no domestic animals are kept.

In the Baliemvalley, traffic is greatly restricted by intertribal warfare. Trade is performed by trader-tribes from outside the valley, the members of which, for selfish reasons, are respected by all parties. Articles of commerce are shells, ornamental feathers, and other commodities of worth in stone-age culture only.

In the Kemaboe and adjacent valleys traffic is more intense because of the presence of a salt well near Homejo. Salt is scarce in the highlands and people walk for days to obtain some. Stems of ferns are soaked the night over in salt water, and consumed as such.

Baliemvalley. The Baliemvalley is an extensive valley at 1,600 m altitude. The valley floor is flat, several km wide, and covered with pools, marshes and numerous ditches for drainage of potato fields. The number of inhabitants is more than 50,000.

In 1938, members of the Dutch-American Archbold expedition were the first to enter the valley. In 1954, an American missionary society settled there; in 1955, the Government.

Toxopeus, of the Archbold expedition, could collect *A. farauti* and *A. koliensis* at 1,700 meters and *A. farauti* at 2,250 m.[9] Both species are punctulatus anophelines and potent vectors of malaria at lower altitudes.[10] Christian[11] proved *A. farauti* to be a vector in the highlands of Australian New Guinea.

Voors[16] was the first to perform a survey in the Baliemvalley. In 60 *A. farauti* no sporozoites were found. Not one spleen was palpable. The results of the blood-examinations are reproduced in Table 1.

From 25th April until 7th May, 1958, there was an opportunity to do a second survey in the valley.

In the evening hours, 210 *A. farauti* and 50 *A. koliensis* were collected on human baits at a rate of 10 per man/hour. On two mornings, eight *A. farauti* and two *A. koliensis* only, were found on the inner walls of native houses. On dissection all were negative.

Bloodslides were collected from native inhabitants living in a stretch of about 20 km along the river Baliem. For the results of the examination see Table 1. In the case of the unidentified parasite, *P. falciparum* could be excluded. Like Voors, I found the positives in the lower parts of the valley only. The difference in altitude between the highest and lowest villages is estimated to be 75 m. Only one spleen could be palpated.

According to the missionaries, the inhabitants never go out of the valley. Some degree of local transmission must, therefore, be accepted.

An observation, of interest with regard to the epidemiology of malaria in the Central Highlands, was made when members of a trader-tribe were contacted, living in still uncontrolled area outside the valley, at a distance of about 4 days by foot. They knew the sago palm, which does not occur above 1,000 m. Several parasite carriers, even of *P. falciparum*, were found among them. The figures are reproduced in Table 1.

Kemaboevalley. At Homeo an American mission post and airfield are made on the steep slopes of the Kemaboevalley, at an altitude of 1,600 m. The salt well is at river level, about 200 m lower,

TABLE 1

Area	Authority	Bloodslides										Spleens percent. palpable
		Number examined	Number pos.	Absol. paras. rate (%)	*P. falciparum*		*P. vivax*		*P. malariae*		Non-class.	
					pos.	gam.	pos.	gam.	pos.	gam.		
Baliemvalley	Voors	653	6	1	0	0	3	3	3	1	0	0
Baliemvalley	Metselaar	450	5	1	0	0	1	1	3	0	1	0.2
Traders-tribe from outside Baliemvalley	Metselaar	41	5	12	1	1	1	0	2	1	1	7
Kemaboevalley	Couvée	73	51	—*	22	19	20	8	4	3	5	—
Kemaboevalley	Metselaar											
Homeo and lower		146	55†	38	23	12	23	5	16	3	0	45
High on slope		33	5	15	1	1	3	1	1	0	0	5
Boegelo on ridge		25	12†	48	5	4	2	0	6	0	0	32
Woebitapa on ridge		14	1†	7	0	0	1	0	1	0	0	0
Whagivalley	van Thiel & Metselaar	119	7	6	6	1	1	0	0	0	0	32
Whagivalley	Black[2]	134	24‡	18	15	—	5	—	4	—	—	74

* Absolute parasite rate not calculated because the slides were somewhat selected.

† With mixed infections.

‡ Gametocytes not mentioned.

the tops of the slopes are at about 1,900 m and higher. A valley floor is practically absent. Small hamlets are spread on less steep parts of the slopes.

Since the mission is working there, fever cases, resembling malaria, were observed occasionally, but the diagnosis was never confirmed by blood examination. Sometimes people, coming from far for salt, fell ill on the way home.

In May, 1958, the mission asked for assistance because of a high number of fever cases and the Government's physician reported an epidemic of malaria with at least 16 deaths in a population group of about 1,000 people. He mentioned a period of unusual drought and the salt well as of possible importance for the origin and spread of the epidemic (Couvée, unpublished report). The results of the examination of the bloodslides (partly selected from patients) are summarized in the table.

Between 20 and 26 August we could collect supplementary information.

The villages, from which inhabitants were seen, can be divided into three groups, namely a group near Homejo and the salt well, on the lower third of the slope; a group higher on the slope; and two villages (Boegelo and Woebitapa) at about 1,900 m on mountain ridges. The malariometric indices found were as summarized in Table 1.

The epidemic had already passed its peak. Few parasite-carriers experienced fever and, for several weeks, nobody had died.

During our visit heavy rains started each day just before sunset and anophelines could not be collected. A few larvae of *A. koliensis* were collected near the airfield and lower on the slope. Neither near the higher slope villages nor on the ridge, could *Anopheles* larvae be found. On the ridges, a few *Culex* larvae were present in breeding places which looked suitable for punctulatus anophelines also.

An unusual dry period must, indeed, be mentioned as the possible cause of the epidemic. Rainfall is, as a rule, moderately heavy all the year round in the Kemaboe valley. The mean monthly precipitation since 1952 is 256 mm, with a minimum of 210 and a maximum of 330. However, between 11 March and 10 May, 1958, half of the normal quantity of rain fell; in April, only 87 mm. It is supposed that for that reason the breeding places on the steep slopes, usually flushed, could now produce abnormal quantities of anophelines. They caused the first cases of the epidemic to appear by the end of May, and a peak in June. Moreover, more sunshine and higher temperatures can have promoted the rapid completion of the extrinsic cycle of the parasite.

Most probably transmission was restricted to

the lower parts of the slopes only. The salt well may have contributed to the spread of the disease. Higher on the slopes significantly lower indices were found than near the river. People living there sleep at home when salt is made. Inhabitants of Boegelo, on a ridge at some distance from the well, usually stay over night near the well when they go for salt making. Their indices are high. People from Woebitapa live on the ridge directly above the well. Though they occasionally stay down, this probably is done less frequently. Therefore, the higher the villages the lower the indices, except those in Boegelo, the inhabitants of which are almost forced to pass the night near the well because of the distance.

For the control of the epidemic, a combination of chloroquine and pyrimethamine was distributed in a single dose (450 and 50 mg, respectively, for adults) to all people which could be reached. In eventual future epidemics, such distributions can be combined with residual spraying. The houses are suitable for it.

DISCUSSION

Before the war it was believed that in New Guinea malaria transmission took place at altitudes below 1,200 m only, and that isolation of population groups by tribal warfare prevented the spread of parasites. When, in 1945, Christian first found malaria parasitss in natives near Mount Hagen (1,650 m) in the Australian part of the island and concluded that malaria was endemic in the area,[13] there was general disbelief.[5] Shortly afterwards Christian found malaria in the Whagivalley at about 1,600 m altitude.[11] Since that time, the opinion that malaria has been endemic in the highlands of Australian New Guinea from times immemorial, is rapidly gaining ground. It finds strong support in the observations described in this paper from Netherlands New Guinea, which make it clear that tribal warfare does not prevent movement of members of trader-tribes over long distances, and which point to the epidemiological importance of a salt well.

In February, 1954, van Thiel and Metselaar (unpublished) were kindly allowed to do a small survey in the Whagivalley, and found much lower indices than Black[2] a few months later (see Table 1). Later, Spencer and Spencer[13] concluded that seasonal variations are a regular occurrence there.

Such variations have, so far, not been observed in the Baliemvalley. The surveys even suggest that in large parts of the valley anophelism without malaria prevails. In other parts the indices indicate a very low degree of transmission, and it can be assumed that the reproduction rate[9] will usually be found below 1. Therefore, malaria should be absent if it were not regularly reintroduced by the autochthonous traders from outside. Under natural conditions the Baliemvalley must be situated at the borderline of the possibilities for malaria transmission. The fact that, so far, *P. falciparum* has not been seen in the Baliem population, though it was found in the traders, supports this supposition. Between January, 1957, and April, 1958, the mean monthly temperature in the Baliemvalley varied between 18.7° C and 20.2° C, the monthly means of the temperatures at noon were between 22.5° C and 25.2° C, while half the number of nights the minimum temperature did not drop below 15° C. It seems that at such temperatures development of *vivax* parasites inside the mosquito-host, though prolonged, is not prevented.[4] The mean monthly relative humidity varied between 52% at noon and 97% at night. The minimum, both of temperature and humidity, was probably higher in the microclimate of the daytime shelter places of the mosquitoes.

Compared with the mean monthly temperatures from the Whagivalley[2] the temperature in the Baliemvalley is on an average 0.5° C lower. This may provide the explanation why malaria is more serious in the former than in the latter.

Souness[11] found malaria in Australian New Guinea at 1,900 m. It is, however, not stated whether the infection could have been obtained at lower altitudes. The observations in the Kemaboevalley made it probable that transmission about 400 m lower, near the salt well, was responsible for parasites found at 1,900 m.

With the scarcity of parasite carriers at 1,600 m in the Baliemvalley in view, notwithstanding the presence of fair numbers of potential vectors and a suitable temperature for completion of the extrinsic cycle, it is very likely that the borderline of transmission is below 1,700 m in that valley. As *A. farauti* has been observed at 2,250 m, there must prevail a zone of anophelism without malaria above the zone of transmission. This may be the fact in more parts of the highlands of New Guinea. It should not create a surprise if, in this island, transmission should seldom, if ever occur above 1,700 m.

Reviewing high-altitude malaria, Bhatnagar[1] states that "the highest limit for malaria incidence almost invariably coincides with the highest

altitude at which breeding of anophelines takes place." Punctulatus anophelines seem to be responsible for one of the few exceptions. Two facts can serve as an explanation, namely relatively short expectation of life and exophily.

The probability of a short expectation of life of punctulatus anophelines was pointed out by Metselaar.[10] At higher altitudes this may be the reason that completion of the extrinsic cycle is seldom achieved, even when the temperature itself should not be an obstacle for this completion.

Most vectors, responsible for malaria at high altitudes, are endophilic. In epidemics at 2,250 m in Kenya[6] development of parasites was possible in *A. gambiae* because its resting places were inside heated human dwellings. Other endophilic anophelines, which could be incriminated as vectors at the altitudes indicated, are *A. superpictus* (2,400 m),[6] *A. fluviatilis* (1850 meter),[8] *A. pseudopunctipennis* (2,600 meter).[7] The preference for outdoor resting places of punctulatus anophelines[14, 15] could be confirmed in the Baliemvalley by the low catches from inner walls.

On the other hand, in some of the cases of transmission at high altitudes, mentioned above, malaria was introduced only recently due to many man-made breeding places as a result of land reclamation.

This can happen in New Guinea also. Christian[11] was the first to warn that, with the march of civilisation, epidemics might occur in the mountains. Spencer *et al.*,[12] recently observed one at 1,650 m near Mount Hagen. That climatological circumstances may also be responsible for outbreaks was suggested by the Homeo epidemic.

However, because of longevity and exophily, it may be expected that the upward extension in punctulatus areas which could be promoted by development projects, will be limited.

SUMMARY

Two malaria surveys in the central mountains of Netherlands New Guinea are described, one of which was carried out during an epidemic. In the Baliemvalley *A. farauti* and *A. koliensis* were collected; in the Kemaboevalley larvae of *A. koliensis*. These anophelines are well known vectors, though their role in transmission could not be proved in the areas concerned. The epidemiological importance of trader-tribes and of a salt well for the spread of the disease in the highlands is stressed. It is suggested that the borderline of transmission in New Guinea is situated below 1,700 m. The possibility that the exophilic habits and a relatively short average duration of life of the anophelines might be an impediment for the spread of malaria to higher altitudes, is discussed.

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ISOLATION OF YELLOW FEVER VIRUS FROM MAN AND MOSQUITOES IN THE AMAZON REGION OF BRAZIL*

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Yellow fever exists in enzootic form in the Amazon Valley. Here, in the enormous forest canopies where favorable ecological conditions are always present somewhere, mosquitoes and monkeys can maintain a perpetual cycle of infection. The epizootic state shifts from place to place as local conditions are modified by the immunization of the susceptible population or the relative abundance of the insect vector. The casual passage of the vertebrate host from one ecological zone to another, or the introduction of susceptible immigrants, may precipitate an epizootic. Infected vectors passing from the enzootic region to disconnected areas where a low percentage of immunes exists in the primate population may likewise spark epizootics, as shown by those which have occurred at intervals in rural central and south Brazil.[1]

The eradication in Brazil of *Aedes aegypti* has resulted in the absence of yellow fever in the cities. Moreover, the extensive vaccination program has reduced enormously the possibility of man's becoming infected in any circumstance. However, there have been sufficient fatal cases in human beings, proven by histopathological examination of liver specimens, to show that the virus is ever-present in the Amazon Valley. Reports on liver specimens by the National Yellow Fever Service for the period 1935 to 1957 record fatal cases in the Valley for every year except 1948 and 1950 to 1954. The discovery of these cases is the more remarkable in view of the vastness of the region and the proportional paucity of viscerotomy posts.

Any susceptible human being who enters the forest during an epizootic of yellow fever is subject to the risk of infection. This risk is increased when (a) his activities disturb the forest canopy so that infected mosquitoes descend to ground levels and have access to him for blood meal, or (b) his presence attracts the monkey band from the canopy to his habitation or the forest clearing where he has planted crops. Epidemics of sylvan yellow fever resulting from both these situations have been studied by the Belém Virus Laboratory in the course of investigations of febrile illness in the environs of Belém. In the Oriboca Forest epidemic only the adult male was involved, which is characteristic of the conditions cited in (a). In the Apeu Forest epidemic the entire family was exposed, which is typical of the conditions cited in (b).

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ORIBOCA FOREST EPIDEMIC

The Oriboca Forest is situated on the Guama River about 20 km east of Belém and forms one of a series of tracts of virgin rain forest that extend both south and east from the city in virtually unbroken succession. The northern limit of the forests is the railroad connecting Belém with Bragança on the Atlantic coast. North of this dividing line the land has been cleared for agriculture in past generations and is thickly settled. South of the railroad, however, population density diminishes sharply, and for hundreds of kilometers beyond the Guama the country is uninhabited except for Indian tribes and isolated communities on the rivers. These areas are populated by the usual forest animals of the region, among which monkeys, sloths, marsupials and rodents predominate. The prevalent monkeys belong to the genera *Alouatta*, *Saimiri*, *Tamarin* and *Aotus*.

In 1954 the Oriboca Forest was selected as the site for a rubber plantation. The opening of trails was started on November 16, and 1 week later the Belém Laboratory began examinations of the laborers. Daily visits were made to the site of operations where temperatures were taken and blood specimens collected from all persons with fever, headache or body pains. Examinations continued until early April, 1955, by which time ten cases of yellow fever had been discovered,

TABLE 1

*Yellow fever patients in the Oriboca Forest epidemic**

Case No.	Virus No.	Date	Temp. °C.	Day of illness virus isolated	Headache	Epigastric pain	Backache	General body pain	Nausea	Vomiting	Photophobia	Vertigo	Icterus	Severity of attack†	White blood count
UR 46	H 68	11.25.54	39.6	3	+	+	+		+	+			+	2	2,800
UR 47	H 70	11.25.54	38.4	3	+	+		+	+	+				2	3,800
UR 48	H 71	11.25.54	37.8	2	+	+						+	+	2	3,600
UR 29	H 96	12.9.54	38.8	2	+	+	+			+				2	4,000
UR 51	H 98	12.9.54	37.8	2	+	+							+	1	5,000
UR 57	H 99	12.9.54	38.9	2	+	+	+	+			+	+	+	3	3,600
UR 79	H 102	12.11.54	39.8	1	+	+								1	
UR 11	H 109	12.15.54	38.4	2	+	+		+			+			3	4,000
UR 19	H 111	12.16.54	38.0	1	+	+								3	5,600
UR 19	H 117	12.17.54	39.4	2	+	+	+			+					4,200
UR 220	H 223	4.1.55	38.2	2	+									1	

* Patients all males, aged 16 to 40 years.

† Classified according to the scale: 1 = mild illness, with rapid recovery; 2 = moderately severe illness; 3 = severe illness with prolonged asthenia.

as proved by isolation and subsequent identification of the virus.

The first two cases were seen on November 22, and a third on the following day. All three patients were working in a section of the forest known to be inhabited by a band of howler monkeys (*Alouatta*). On December 9 three more cases were found, and an additional one on each of the three days December 11, 15 and 16. During the rest of December and the first quarter of 1955, 110 laborers were examined, but no further cases of yellow fever were found. This cessation of transmission of yellow fever was undoubtedly due in part to the fact that many of the monkeys had retreated from the area because of the profound disturbance of their habitat. Once operations were under way, the howlers were never heard in the vicinity, although trail blazers reported hearing these or another band deeper in the forest toward the Guama River. The *Saimiri*, which also were abundant before the work began, were likewise neither seen nor heard after the first few days. The night monkeys (*Aotus*) were encountered occasionally during all the cutting operations, and several were captured in felled hollow trees; presumably they remained in the area because there were no unusual noises in the forest during their active hours.

Early in March the Yellow Fever Service vaccinated all workers then present in the forest as well as a large number of residents in the adjacent counties. Subsequently new laborers were introduced, one of whom contracted yellow fever. He and several companions were employed in opening a trail about 6 or 7 km from the site of the November operations, in a part of the forest toward which the *Alouatta* monkeys were known to have retreated.

Clinical data on these ten cases of yellow fever are listed in Table 1 together with observations made on the occasion of bleeding for virus studies. White blood counts made on eight cases on the first to third days of fever were all low; the one case (UR19) examined on both the first and second days of fever showed a fall from 5,600 to 4,200. White counts made on two patients during convalescence were 9,600 (UR46) and 11,200 (UR19). The common complaints were severe headache, which generally outlasted the fever, and epigastric pain. Backache, especially over the kidneys, and muscle and joint pains were reported, as well as nausea, vomiting, vertigo and photophobia. Anorexia and asthenia were generally noted. Light icterus was observed in four patients. The severity of illness was objectively classified as (1) mild, when fever was of short duration followed by rapid recovery and little or no malaise; (2) moderately severe, when the febrile state was followed by a period of continued pain and obvious asthenia; and (3) severe, when the second stage and the period of

asthenia were both intense and prolonged. According to this scale, there were three mild, four moderately severe and three severe cases in the Oriboca epidemic. All patients recovered, and no deaths occurred among the forest workers during this period. Although each of the ten laborers returned to his home while still febrile, there were no secondary cases in any of the families or neighbors.

The viral agents were identified as yellow fever by the New York Virus Laboratories of The Rockefeller Foundation.[2] Paired acute and convalescent serum specimens from each patient showed conversion from negative to positive for yellow-fever-neutralizing antibodies.

Yellow fever virus was also isolated from four pools consisting of 270 diurnal mosquitoes collected in the Oriboca Forest between December, 1954, and the end of April, 1955. These mosquitoes represented about 1% of the total specimens captured in the forest and inoculated in attempts to isolate virus during this period. The first isolation, AR154, was made on January 21 from a pool of 6 *Aedes* and 49 sabethine mosquitoes. The second, AR162, was made on January 26 from a pool of 12 *Haemagogus*. The third virus, AR189, was obtained from a pool of 177 sabethines collected on February 9. Another strain of yellow fever virus, AR245, was isolated from a pool of 26 *Haemagogus* caught on March 9.

APEU FOREST EPIDEMIC

The village of Apeu on the Bragança Road is about 60 km east of Belém. Here the forests that border the River Apeu are an extension of the Guama River forests of which the Oriboca is a western extremity. The inhabitants along the Apeu are truly forest dwellers. Contrary to the usual custom on larger rivers, they do not clear the land close to the houses but go inland several hundred meters to where the forest stand is more uniform and easier to cut for charcoal, the principal source of income for many families. As clearings are opened, rice, beans, corn and mandioca are planted. The clearings are small and are worked by family groups; even the tiny children take part in the daily chores. *Saimiri* monkeys are numerous in the vicinity of the houses and around the fields, where they share the fruits of man's labors.

Late in April, 1955, reports reached the laboratory from Apeu that fever cases which did not respond to malaria therapy were occurring in alarming numbers. Investigation of the epidemic was begun on the 26th and continued until May 26, when an extensive vaccination program was instituted in the region. Of a total of 105 sick persons bled for mouse inoculation, 11 yielded the virus of yellow fever.[2] These cases are listed in Table 2 with clinical data and observations made at the time of bleeding. It can be seen that all age groups were involved and that a surprisingly large proportion were adult males, who might have been expected to be immune because of their occupation.

Of the remaining 94 persons examined, one died on April 28 with classical symptoms of yellow fever, and two others from whom no virus was

TABLE 2

Yellow fever patients in the Apeu Forest epidemic

Case No.	Virus No.	Date	Temp. °C.	Day of illness virus isolated	Headache	Epigastric pain	Backache	General body pain	Nausea	Vomiting	Photophobia	Vertigo	Icterus	Age	Sex
AP 7	H 314	4.26.55	37.7	3	+			+			+	+		35	M
AP 22	H 337	5.2.55	39.4	3	+					+		+		5	M
AP 24	H 339	5.2.55	38.1	1										9	F
AP 55	H 366	5.4.55	39.1	3	+				+			+		30	F
AP 51	H 362	5.3.55	37.5	4						+				12	M
AP 53	H 364	5.4.55	37.3	2	+	+				+				12	M
AP 66	H 377	5.4.55	37.4	3		+			+					44	M
AP 67	H 378	5.5.55	39.1	4	+		+	+	+			+		20	M
AP 96	H 448	5.20.55	38.0	3	+		+							18	M
AP 97	H 449	5.20.55	39.8	2	+				+			+		8	M
AP 100	H 452	5.23.55	38.1	3								+		14	M

isolated showed conversion from negative to positive for yellow fever antibodies on examination of acute and convalescent serum samples. A further 23, with no record of vaccination for yellow fever, showed neutralizing antibodies in the first blood sample taken; some of these may have been recent cases, since several of the patients gave a history of illness and prolonged convalescence not unlike that of the yellow fever patients. In all, 36 persons, or 50.7% of the 71 examined serologically, yielded virus or showed protective antibodies. An additional yellow fever case that escaped investigation was reported by the viscerotomy service in the routine examination of fatal febrile illness of short duration.

SUMMARY

An epidemic of jungle yellow fever was investigated in November and December, 1954, and early 1955, in the Oriboca Forest about 20 km distant from Belém, Pará, Brazil. The virus was isolated from ten persons all of whom were employed in opening trails in a virgin forest.

A second epidemic was studied near the village of Apeu about 60 km east of Belém in April and May of 1955. Yellow fever virus was isolated from 11 cases.

A comparison of the acute and convalescent sera of all 21 cases showed a significant rise in antibody titer for yellow fever virus.

Four strains of yellow fever virus were isolated from mosquitoes caught in the Oriboca Forest at the time of the jungle epidemic.

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EPIDEMIC JUNGLE FEVERS AMONG OKINAWAN COLONISTS IN THE BOLIVIAN RAIN FOREST

I. Epidemiology

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INTRODUCTION

In the summer of 1955 the devasting effects of a prolonged outbreak of febrile illness associated with high mortality spelled doom to a valiant attempt of Okinawan colonists to pioneer a new settlement in the lowland rain forests of eastern Bolivia. The inability of modern medicine to overcome the hazards of "jungle fever," long recognized as one of the great deterrents to colonization of the tropical jungles of the world, was clearly emphasized in this failure of a carefully planned, mid-Twentieth Century pioneering venture. Furthermore, study of the epidemic throws into clear focus the current inadequacy of medical knowledge and facilities to foresee, prevent or control a similar catastrophe in future attempts to settle the vast, still unproductive jungle lowlands. This report deals with the epidemiology of the epidemic which plagued the struggling colony from shortly after its inception to its final abandonment and with the isolation of a virus responsible for some, but certainly not all, of the cases of "jungle fever" which occurred.

HISTORICAL BACKGROUND

Okinawa, of the Ryukyu Islands, overpopulated for centuries, has suffered from population pressure, particularly since the close of World War II, when many natives returned to the islands from Japan and Manchuria and when the establishment of American installations on the islands further curtailed the available farming lands, already encroached upon by Japanese military bases. In an attempt to resettle the many Okinawans who wished to emigrate from the islands, a world survey revealed few available sites for colonization by these agrarian people, because of such factors as restrictions on immigration of Asiatic nationals or lack of available land which could be released to them as freeholdings. A survey by James L. Tigner, of the University of Southern California, of previous Okinawan emigration to South America revealed scattered small groups of Ryukyu islanders throughout the continent and suggested the Santa Cruz Area Development Program (SCADP) in the *llanos*, or lowland plains, of eastern Bolivia as a promising site for Okinawan resettlement in a region where the Bolivian government would welcome their arrival.[1]

This area around the colonial city of Santa Cruz had been independently selected as the site of a vast settlement program aimed at overcoming the problem of Bolivia's long standing deficiency of foodstuff production. Internal migration from the densely populated altiplano and immigration of people from other countries to the Santa Cruz area were both encouraged and supported by the Bolivian government, by the United Nations program of assistance to Bolivia and by the United States Operations Mission assisting the Bolivians in the American foreign aid program. The Santa Cruz area contained uncultivated land of sufficient size and acceptable climate and soil conditions to produce, in a short period of time, practically all food for which Bolivia was currently expending sorely needed

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foreign exchange, and could yield in addition a surplus for export.[2] A trucking road from Santa Cruz (elevation 1,575 feet) across the mountainous and forested *yungas* and *valles* region, which separates the sparsely settled lowland plain from the highly-populated altiplano, or high plateau, had already been constructed as part of the SCADP, and there was a rail connection eastward to the Brazilian coast interrupted only at the Rio Grande, or Guapay, 50 km east of the city. This river might eventually be bridged to provide a direct outlet to the sea for exportable produce.

The site picked for the first Okinawan colony, named Uruma, was an unfortunate choice. Through circumstances no longer discernible, the location chosen was far outside the area surveyed, studied and proposed for colonization in the SCADP. Instead of lying in the project region between the Rio Pirai, which flows through Santa Cruz, and the Rio Grande some 50 km to the east, it lay in the unexplored forests east of the Rio Grande, in jungles which were inhabited only by hostile, uncivilized Yanaigua Indians who have never been studied anthropologically nor brought under mission influence.

Uruma, the place chosen for the colony, lay in the Province of Nuflo de Chavez, 6 km east of the Rio Grande on the right bank of the river, which is a tributary of the Amazon River, and some 37 km north of Puerto Pailas, where the Corumba-Santa Cruz rail from Brazil ends at the unbridged, and not easily bridged, Rio Grande. Uruma is some 60 airline km northeast of Santa Cruz and lies in a region without vehicular roads. Access was via jungle tracks adequate for horses, bullock carts or, in the dry season, vehicles with four-wheel drive. Many portions of these primitive paths are flooded every year. In fact, during the rainy season (January through April) the water of the Rio Grande spreads many kilometers beyond its banks and, penetrating into the jungles, often reaches a depth above 1 m over a wide area. Rising water from such flooded jungle extended into the clearing of the Uruma settlement to inundate all but the higher ground of the colony site which became a virtual island.

Two Okinawan families from Santa Cruz, who immigrated to Bolivia many decades ago, had lived on this site in an attempt to pioneer a new frontier, but they had abandoned it because of attacks by the hostile Indians who had killed one of their members. In June 1954, under the leadership of an old Okinawan resident of Santa Cruz who was the leader of these earlier Ryukuan immigrants to Bolivia, 63 workers hired from Santa Cruz arrived at the prospective site to clear the forest, plant crops and construct houses for the colonists. This group stayed at Uruma until November 30, 1954.

The first part of Okinawan colonists, carefully picked from 40,000 volunteers to form a well-prepared pilot group of healthy pioneers, travelled from Okinawa to Santos, Brazil in the summer of 1954, touching the African coast en route. These immigrants, numbering 270, arrived at the Uruma colony site on August 13, 1954, during the dry season, and the second group of 130, mostly women and children, arrived on September 15, 1954. The month of ocean voyage had been followed by another month of overland journey on primitive rail from the Brazilian coast to the heart of the South American continent. At the rail termination on the east bank of the Rio Grande, they left the train to trek 37 km northward along a recently-cut jungle track to the Uruma site.

Before leaving Okinawa in mid-May, 1954, all the immigrants were immunized against smallpox, typhoid, and epidemic typhus. On November 5, 1954, they were vaccinated against yellow fever by Bolivian medical personnel at Uruma, at which time all the children were given their first injection of triple vaccine (tetanus-pertussis-diphtheria); second injections were given a month later.

Although there had been some minor illness among the colonists while travelling to Bolivia, they arrived at the colony site in good health. They started to work immediately with the indigenous crew, composed of Indians and others of mixed blood hired from Santa Cruz. Since only incomplete preparations had been made for their arrival, house building, planting and further clearing of the forest were their principal tasks.

Severe febrile illness first appeared among them in late October and early November when three adults became ill in rapid succession; the first case, in a man of 45 years, dying on the third day of his illness. From that time until the colony was abandoned in July 1955, the outbreak never completely subisded.

ECOLOGY AND PHYSICAL SITUATION

Geographical Location. A map locating the Santa Cruz region in South America and a larger

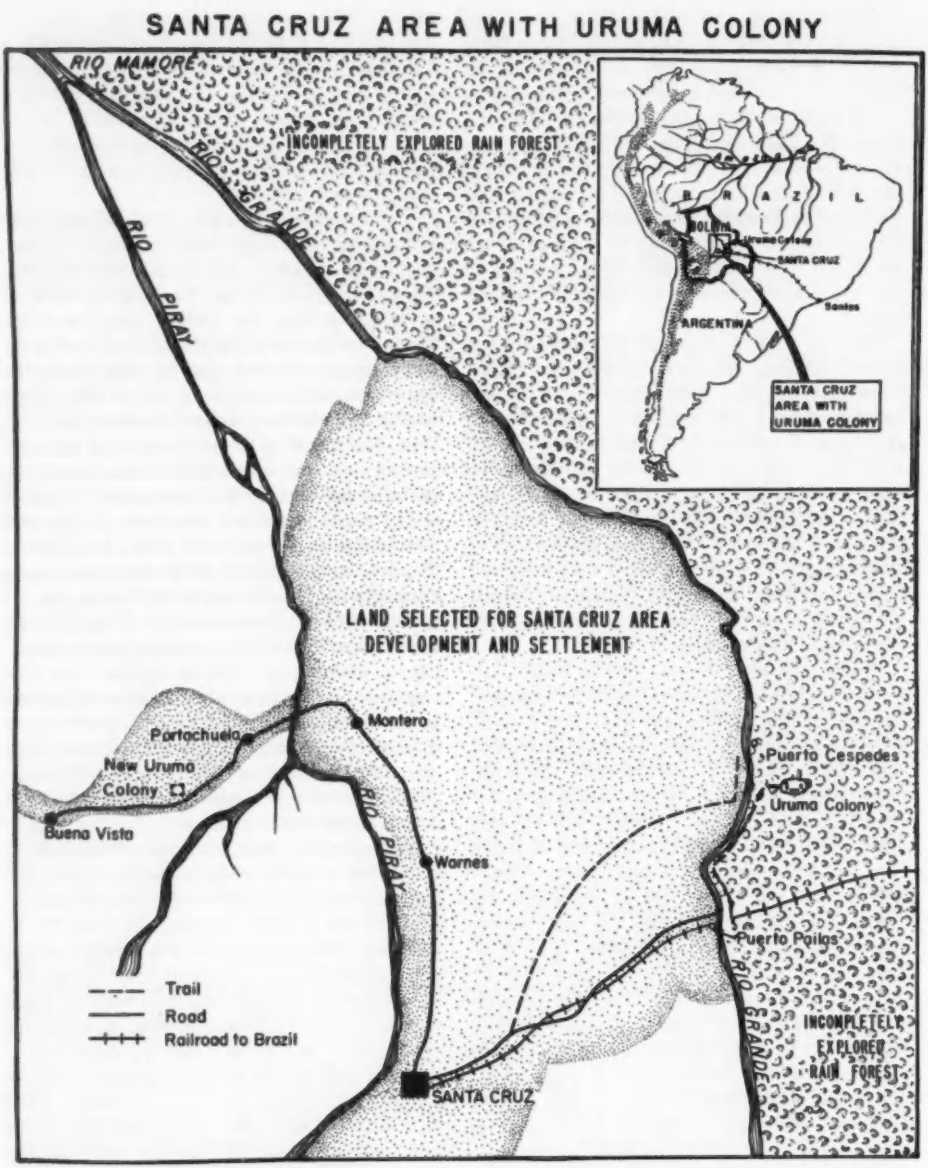

FIG. 1. Location of the Santa Cruz Area in Bolivia (insert). The larger scale map shows the area selected for development and settlement. The Uruma colony is shown located in the incompletely explored jungles to the east of the Rio Grande.

scale map showing the Uruma colony site and the city of Santa Cruz are given in Figure 1. Further geographical details have been presented in the previous section. The nearest settlement of civilized Bolivians to the Uruma colony was a small settlement of indigenous, mixed-blooded Indians and Caucasians about 12 km away on the eastern Rio Grande shore to the north of the Okinawan colony. The residents of this settlement, Puerto Cespedes, were subsequently included among subjects of a recently reported serological survey of the upper Amazon Drainage of South America.[3] Many Puerto Cespedes people visited Uruma from time to time. The only other civilized settlements within a wide radius lay on the west side of the river which in wet season provides a very formidable barrier and is crossed only with the help of local inhabitants manning a large, flat-bottomed boat, which they paddle with skill, in the swift and treacherous water.

Climate and Rainfall. The low, dense forest of the Uruma region lies on a fertile, sandy soil covered with lush vegetation. It is flooded annually in the rainy season (January through April) when torrential rains fall daily. Average daytime temperature varies between 24°C and 27°C, with sharp declines to about 10°C at night or accompanying strong winds from the south. These southernly winds are dominant but strong north winds may prevail from August through November at midday. In winter, temperatures do not drop to freezing, but a low of 4°C may be reached.

Housing. A plan of the Uruma settlement is presented in Figure 2. The colonists lived in three barrack-like buildings constructed of palm thatch, dried mud-clay and wooden beams. All

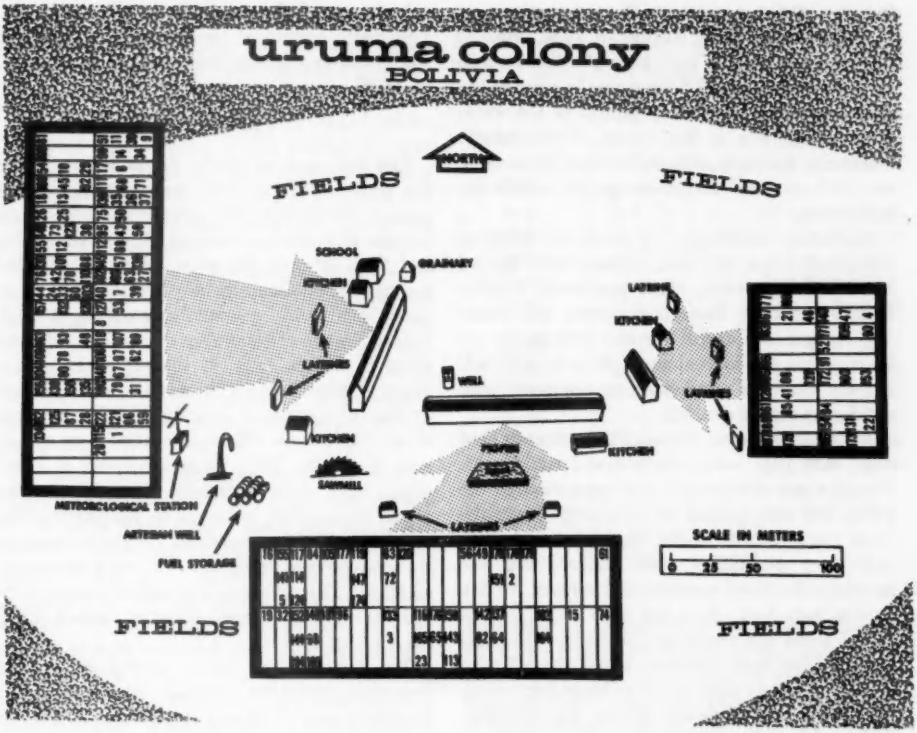

FIG. 2. Plan of the Uruma colony showing the three large, barrack-like residences. The cases numbered in chronological sequence of occurrence are plotted as to place of residence at the time of onset. The three cases occurring in colonists after leaving the colony for Santa Cruz are not indicated.

barracks were equidistant from the border of the jungle, approximately 300 m away. Two were 100 m long and the third about half that length. These were called the West, South and East Buildings. Lengthwise central partitions bisected each barrack, and the two halves were each divided into 23 living quarters in the West and South barracks and ten in the East. Each of these 46 (or 20) living quarters per barrack measured 4 x 3 m with walls about 2 m high which did not reach the ceilings. The floor was an Okinawan-style living platform about 1 ft above the ground, used as a kitchen, eating table, sitting room and bed for the entire family. Each family did its own cooking, with the food drawn from a common central supply. The family slept together on the Oriental-style platform under one large mosquito net. The community was closely knit and the various families mingled freely. Children from all three barracks played together. There was, therefore, almost constant contact between all the members of the community.

Water Supply. Until November, 1954, drinking water was carried in jugs from the Rio Grande or from a small lake 1.5 km away because of the excessive salinity and poor quality of the water from a well dug in the center of the colony. Thereafter a deeper well, drilled over 80 m deep, provided water of acceptable quality within the colony site.

Sanitation. Although standards of personal cleanliness were high and clothes were washed often and kept clean, there was never a satisfactory system of disposal of excreta and waste. The latrines consisted of dug pits covered only by loose boards and located about 30 m behind each barrack. Garbage was heaped in uncovered piles within the colony clearing.

Wild and Domestic Animals. The colonists kept dogs, cats, pigs, oxen, chickens and wild ducks. The pigs and chickens did not roam through the village but were penned. In an attempt to supplement the diet, wild ducks, pigeons, turkey hens and giant armadillos were hunted, and the colonists also killed monkeys and parrots. No live parrots were kept. Rats and mice were plentiful in and about the colony and in the thatched barracks. A few dead rats were found sporadically, but no epidemic among the rodents was noted. No illness was observed among the domestic animals.

Nutrition. The Okinawans' crops, if they were not ruined by excessive drought in one season or inundation in another, consisted of yucca, corn, and rice. With crop failure it was necessary for the government to provide supplementary supplies of flour, milk, butter, sugar and edible oil. Meat was obtained by community sharing of occasionally slaughtered pigs and fowl and whatever the hunters could add to the fare. Diet, while simple, appeared essentially adequate Thus, no cases of malnutrition appeared nor were simple or multiple vitamin deficiencies observed among the colonists.

Arthropods. Insect pests of many sorts on crops, animals and man were varied and numerous. Chiggers and ticks, flies, phlebotomus, and mosquitoes were all found in the colony site. *Aedes aegypti* was not found among any of the mosquitoes collected in Uruma, all of which were found to be culicine. *Culex quinquefasciatus* predominated. Flea and louse infestation was minimal, and none could be collected from the colonists in February and March, 1955. No systematic control of insects by DDT spraying was available or attempted before the onset of the epidemic.

EPIDEMIOLOGY

The outbreak of febrile illness began in the dry season at the end of October and in the first week of November, 1954, when three individuals became ill in quick succession. At this time there had been no rain for several months and the mosquito population was very low. The number of cases then built up rapidly, reaching a peak toward the end of December, declining slowly and rising to a peak again at the end of February. Figure 3 presents the total recorded cases plotted by date of occurrence. Among a total population of 403 individuals 192 cases occurred; an attack rate of 47.6%. There were a number of other people who gave a history of symptoms too vague to warrant inclusion in this survey but which may have represented very mild cases of the same disease, or diseases. Recorded cases were all febrile. Since afebrile, clinically-inapparent infections may well have occurred, attack rates are minimal values.

The rainy season began toward the end of December, when the outbreak was already well developed, and continued through the spring. In early summer when the last of the colonists were finally transferred to their new site on the Rio Palometillas, between Portachuelo and Buena

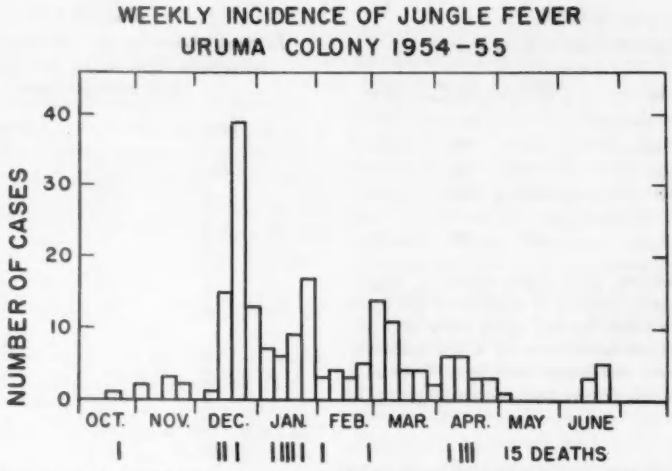

FIG. 3. Distribution of cases of jungle fever by date of onset. The 15 deaths in the colony are recorded by vertical lines below the time axis.

Vista, west of Santa Cruz, the rains had ceased and the floods subsided. Cases continued to occur, however, until the last colonists left Uruma.

The shape of the incidence curve could not be correlated with changes in the weather or with any recognizable changes in the habits or working conditions of the colony. No shift in age or sex distribution of the cases occurred during the course of the outbreak.

The distribution of cases among the two sexes was similar, 132 cases occurring among males and 60 among the females, giving attack rates of 52.0% and 40.3%, respectively. Sex- and age-specific rates also fail to reveal any significant differences, except that children below ages 5 years were less often affected than any other age group (Table 1).

In attempting to interpret these data, it is recalled that all males over age 10 generally worked in the fields while most females did not. It is, therefore, unlikely that the disease was acquired while working in the fields or while clearing the jungle. Rather, it appeared that people became infected in Uruma itself. Furthermore, the frequency of cases occurring among individuals who had not left Uruma since their arrival was identical to that in those who had often travelled back and forth between Santa Cruz and other areas and Uruma.

Figure 2 is a plan of the Uruma colony showing the three large barrack-like residences. The cases, numbered in chronological sequence of occurrence, are plotted as to place of residence at the time of onset. The total numbers of residents in the East, South and West Barracks at the onset of the epidemic were 83, 161, and 165, respectively. Table 2 presents data on case incidence in these three barracks. It will be noted that over half the cases occurred in the West Barracks, and

TABLE 1
Attack rates by age group and sex

Age group	No. of cases		No. of inhabitants		Attack rate (%)	
	male	female	male	female	male	female
0–4	9	8	41	28	21.9	28.6
5–9	8	4	18	15	44.5	26.6
10–14	5	2	12	5	41.6	40.0
15–19	11	4	20	14	55.0	28.6
20–24	40	17	53	20	75.5	85.0
25–29	14	9	40	25	35.0	36.0
30–34	13	6	26	14	50.0	42.8
35–39	16	1	20	7	80.0	14.3
40–44	4	2	5	5	80.0	40.0
45–49	6	2	10	5	60.0	40.0
>50	6	5	9	11	66.7	45.5
Totals by sex	132	60	254	149	52.0	40.3
Both sexes	192		403		47.6	

TABLE 2

Residence of patients in Uruma

Location	Population*	No. of patients	Attack rate (%)
South Barracks	161	52	32.3
East Barracks	83	34	41.0
West Barracks	165	103	62.4
Totals	409	189†	46.1

* As of October 1954, when outbreak began. Some subsequent shifting of residences did occur and therefore these figures apply only approximately during the latter months of the epidemic.

† Three cases developed outside of Uruma in colonists who left during the incubation period of the disease.

that the attack rate in this house is almost twice that of the South Barracks. No explanation for these differences is apparent. The houses were all exactly of the same construction, the rooms of the same size and similarly equipped, the water from the same well, the sanitary facilities identical, as were the sources of food, method and location of food storage, etc. The age and sex distribution of the population in each barracks was almost identical.

Inspection of the case distribution as plotted in Figure 2 reveals that the first case occurred in the West Barracks but subsequent cases rapidly appeared in the others. More detailed analysis fails to show any significant differences between the temporal relationships of cases from the three barracks.

Analysis of the occurrence of illness in those families in which multiple cases occurred (over one third of all family groups) reveals that the secondary cases occurred with an attack rate among the susceptibles in these families which is almost identical to the overall attack rate in the community.

Table 3 shows the intervals between the onset of the index case and subsequent cases in a group of families with multiple cases. The distribution appears to be entirely a random one, indicating that unless a tremendously variable incubation period or period of infectivity exists, the index cases did not infect the subsequent cases.

A total of 15 deaths occurred. These were spread through the epidemic; three occurred in children (of ages 2, 5 and 12 years, respectively) and 12 in older patients. There were no significant

TABLE 3

Interval between dates of onset of index and subsequent cases in a group of families with multiple cases

Interval	Subsequent cases
Days:	
0	1
1	0
2	0
3	1
4	1
5	1
6	0
7	0
8	2
9	3
10	0
11	0
12	0
13	0
14	0
15	2
16	1
17	0
18	0
19	0
20	0
21	0
22	0
23	2
24	1
25	0
26	1
27	0
28	1
Weeks:	
Fifth	6
Sixth	4
Seventh	2
Eighth	1
Ninth	3
Tenth	4
Eleventh	0
Twelfth	1
Thirteenth	6
Fourteenth	0
Fifteenth	0
Sixteenth	1
	45

age or sex differences between the mortality rates.

CLINICAL PICTURE

The disease was an acute febrile illness with no pathognomonic signs or symptoms which would permit unequivocal differentiation from other fevers of unknown etiology. Thus, it was typical "jungle fever" with no diagnostically specific features, although the majority of cases did share certain characteristics. These were:

a. Sudden onset of fever (usually 38.5° to 39.5°C) which lasted 3 to 7 days.

b. Headache, usually frontal and of moderate severity, and lasting 2 to 3 days.

c. Profuse sweating.

d. Polyuria which usually preceded the onset of fever by about 12 hours and continued 1 or 2 days after the onset.

e. Tachycardia out of proportion to the fever.

A typical case of this jungle fever was marked by sudden onset of fever, with such nonspecific prodromata as vague muscle-aches, insomnia, easy fatiguability and mild anorexia preceding the fever by about 1 day. Most patients experienced a true chill with the onset of fever, but chills did not recur. Accompanying the fever were frontal headaches, backaches usually referred to the lower lumbar area, general malaise, moderate anorexia, and mild aches in the extremities. There were no consistent symptoms referable to the gastrointestinal tract. In 10% of the cases, nausea and vomiting were present; when these were most intense, prognosis was at its worst. Hiccoughs were a sign of poor prognosis. Of the 15 dead, six had suffered from this persistent symptom. No photophobia, stiff neck, mental confusion or convulsions were reported. None of the patients was noted to be jaundiced or to have specific skin lesions although in 3% to 4% of the cases a mild, transitory exanthem in the form of a macular erythema appeared on the 3rd to 6th day of illness. No suspicious primary eschar or other evidences of insect bites were noted. The polyuria, of up to 3 L in 24 hours in the majority of cases, was not accompanied or preceded by any appreciable change in fluid intake or by any obvious edema, and no dysuria, pyuria, or hematuria ever developed, although occasional transient albuminuria was observed.

During the acute illness blood pressure was generally normal, but it was low in a few patients. The pulse was regular and fast, averaging about 140 per minute. Respirations were as high as 40 per minute in some patients, but in most patients rose to only 28 per minute.

On physical examination, a moderate, nonpurulent conjunctivitis was sometimes found. Some patients had moderate, generalized, nontender lymphadenopathy. Only a few patients had abdominal pain, aggravated by deep palpation. Diarrhea rarely appeared, whereas constipation was common. There was usually no enlargement of the liver or spleen and no tenderness of these organs nor in the renal areas. Some patients suffering from mild cough had findings on auscultation suggestive of bronchitis and a few developed signs of pneumonitis. The fever curve showed irregular spikes usually reaching a peak of 39°C to 40.5°C on the 2nd or 3rd day and then gradually diminishing. Aside from marked weakness, no new symptoms were noted as the illness progressed.

The average duration of the acute illness was about 1 week, with the period of convalescence averaging about 10 days, but ranging from 3 days to 1 month. Weakness and insomnia were the only complaints during convalescence and no sequelae were observed. In cases with fatal outcome death occurred between the 3rd and 8th day of illness, at times during a fever spike. Even fatal cases did not show coma, disorientation, or convulsions before death. From the clinical picture it was not possible to draw any conclusions as to the etiology of the illness.

Typical Cases. The following two case histories, accompanied by the charts of the patients (Figure 4), illustrate rather typical clinical courses.

Case 1. Y. S., from whom the Uruma virus was isolated

Y. S. was a 28-year-old married woman who had not been out of the Uruma colony site for over 1 month. Two weeks previously her husband was a victim of jungle fever. He ran a typical febrile course with some generalized lymphadenopathy, some cough and a mild, transient pneumonitis. He recovered in 1 week and had been well without sequelae for about a week when his wife first became ill.

She began to feel slightly ill 2 days before she was seen by the authors in mid-March, 1955. Her appetite decreased, she felt achy and did not sleep well. The following day she had a frontal headache for several hours. On the day of examination she noted that she was passing more urine than usual,

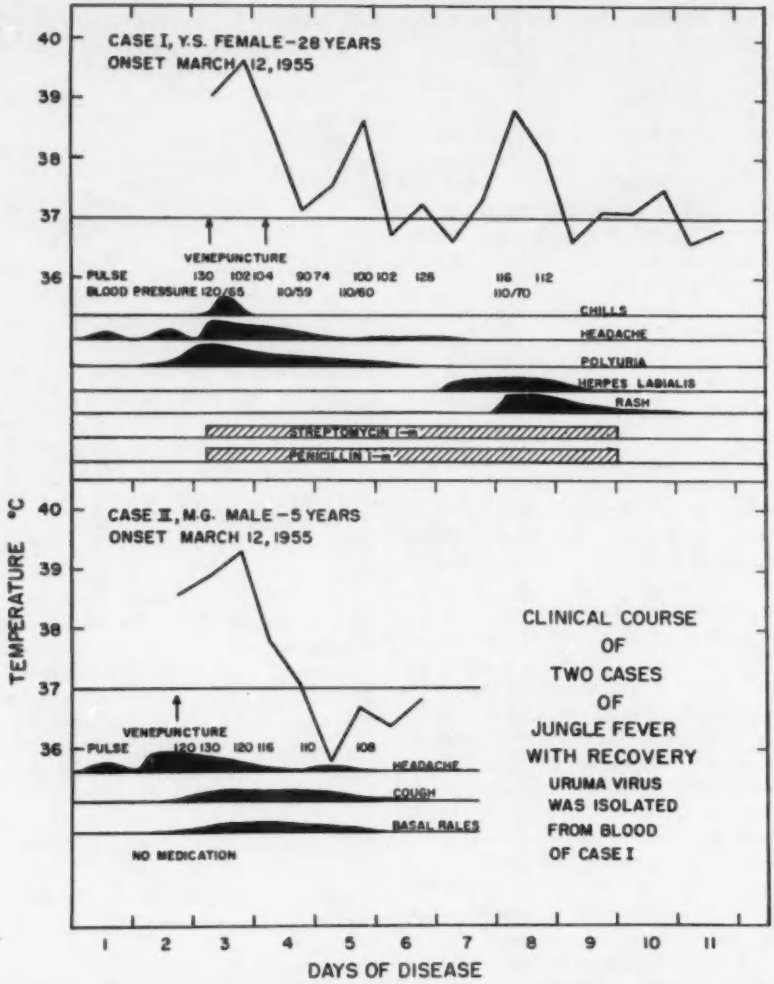

FIG. 4. Clinical course of the two cases of jungle fever. Case 1 is the patient from whom the Uruma virus was separately isolated, from blood taken at each of the two indicated venepunctures.

voiding four times in a period of about 8 hours. There was no dysuria or change of color in the urine. About noontime she began to have an aching sensation in her lower back and her frontal headache returned more severely than the previous day. She began to feel weak, suffered generalized malaise and total loss of appetite. No cough, nausea, or diarrhea developed.

Physical examination revealed an acutely ill, well-nourished woman who was sweating profusely. She was well oriented and complained of frontal headaches; temperature 39°C; pulse 110/minute; respirations 32/minute; blood pressure 110/68 mm. Except for mild conjunctival injection there were no remarkable findings. No enanthem, exanthem, or insect bites were found. There was no lymphadenopathy. There was no drowsiness or disorientation, and no meningismus was present. Opthalmoscopic and neurological examinations were normal. Cardio-respiratory and gastrointestinal systems appeared normal. The patient was given intensive intramuscular aqueous strep-

tomycin and penicillin therapy for 1 week from the day of onset of her fever. In spite of this treatment she continued to run an erratic febrile course. Her temperature reached 38.5°C on the 3rd day of fever and 39.0°C on the 6th febrile day when a mild, generalized, maculopapular erythema appeared on her trunk, extremities, neck and head without predilection in localization. It faded completely within 2 days. Her pulse remained elevated, usually over 100/minute, for a full week and then returned to normal as she became permanently afebrile.

On the 3rd day of her symptoms, the first day that fever was noted, blood specimens were taken for virus isolation and serology at the time her temperature was first taken and found to be 39°C. An additional blood specimen was collected early in the morning of the 2nd day of fever. From both the first and second specimens a virus was isolated, which we tentatively call Uruma Virus. The isolation of this agent is discussed more fully under the section on ETIOLOGY and in a subsequent report dealing with the characterization of the virus.[4]

Case 2. M. G.

M. G. was a boy of 5 years who had not left the Uruma colony in recent months. He was well until following a short period of mild listlessness when he lay down complaining of a headache. There was no cough, nausea, vomiting or diarrhea, and no polyuria or other symptoms were noted.

Physical examination revealed a well-developed and well-nourished, acutely ill boy of 5 years, who was febrile but well oriented. His temperature was 38.4°C.; pulse 100/minute; respirations 28/minute; blood pressure not obtained. A smooth, nontender liver edge could be felt just at the right costal margin and the spleen tip could just be palpated. There was no rash or enanthem, no coryza, conjunctival injection or lymphadenopathy. Ears were clear. Lungs were clear and the heart normal.

The boy remained constantly febrile until the third day, his temperature reaching 39.3°C on the second day. He had a tachycardia of 120 per minute or higher during the febrile course, and as his fever subsided, his pulse only returned more slowly to normal. During the illness he developed a mild cough and on auscultation a few moist rales were detected in both lung bases, but these disappeared with his cough when his temperature returned to normal. He was given no antibiotics or other medication. His recovery was rapid and complete and without sequelae.

Charts of three fatal cases are shown in Figure 5. Few further clinical data are available on these patients.

THERAPY

The disease did not respond dramatically to the administration of sulfonamides, penicillin, streptomycin, chloramphenicol or tetracycline. Neither did quinacrine, amodiaquin, pamaquine or other antimalarials have any significant effect on the course of the disease. In a number of cases nonspecific therapy with such drugs as calcium gluconate and ascorbic acid was also without avail. Because the possibility of malaria was given prominent consideration, some of the local observers believed that over-treatment, especially with the antimalarials, may have accounted for some of the fatalities.

CLINICAL LABORATORY STUDIES

As the number of cases mounted, an expedition from the Bolivian Department of Health arrived at the colony with simple laboratory equipment which made possible a limited amount of clinical laboratory work such as urinalysis, hemoglobin determinations, blood-cell enumerations, examination of blood smears for differential counts and parasites, etc. Such examinations on several dozen acute cases failed to show any significant deviations from the normal. An occasional patient showed a moderate leukocytosis. A number of patients were said to have shown a 1- to 2-plus albuminuria during the polyuric phase but no albumin was found in any of the three acute cases examined by the United States team at Uruma.

Sero-agglutinations on selected convalescent serum specimens were performed at the laboratory at Cochabamba or La Paz and were negative for typhoid, paratyphoid, brucellosis and tularemia.

Although the clinical picture did not suggest malaria, the blood from numerous patients in the acute phase of their illness was examined for malarial parasites in thin and thick film preparations. These were invariably negative with the exception of a few equivocal slides which were not retained by the original observers for verification. Most of the colonists were on irregular antimalarial prophylaxis.

Additional laboratory studies concerned with establishing the etiologic agent of the "epidemic" were conducted in the Virus and Rickettsial Disease Laboratories of the United States Communicable Disease Center and Walter Reed Army Institute of Research, and are described under the section on ETIOLOGY, below.

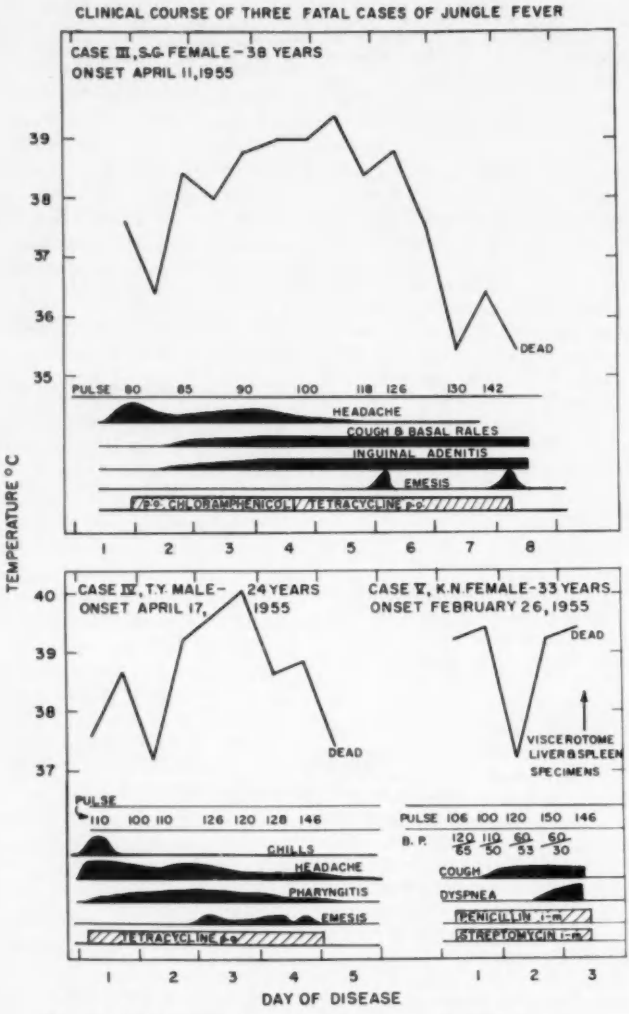

FIG. 5. Clinical course of three fatal cases of jungle fever

PATHOLOGY

The pathologic manifestations must remain obscure since autopsies could not readily be performed because of the resistance of the colonists on religious and cultural grounds. However, small specimens of liver and spleen were obtained by viscerotome from two individuals who died early in the epidemic. The liver specimens were examined at the Oswaldo Cruz Institute and the spleen at the University of San Andrea. Both liver specimens were reported as being negative for yellow fever but lesions of "serous hepatitis and extraneous spiralled organisms morphologically identified with leptospiras" were reported in one of the specimens. The spleen from the other patient was said to show "acute lesions of malaria." These reports on viscerotome specimens are unfortunately uninterpretable.

Later in the outbreak two individuals with prominent adenitis succumbed. Exudate from

FIG. 6. One of the main streets of Santa Cruz, capital city of the Santa Cruz Department of Bolivia, the center of the colonization, settlement and development program for the tropical lowlands of Bolivia.

punctures of enlarged nodes were examined in the Central Laboratory of La Paz but no organisms were identified in the smears.

Thus, with no thorough gross or microscopic post-mortem examinations or any preserved tissue specimens there is no pathological information which might aid in establishing the etiology of any of the cases.

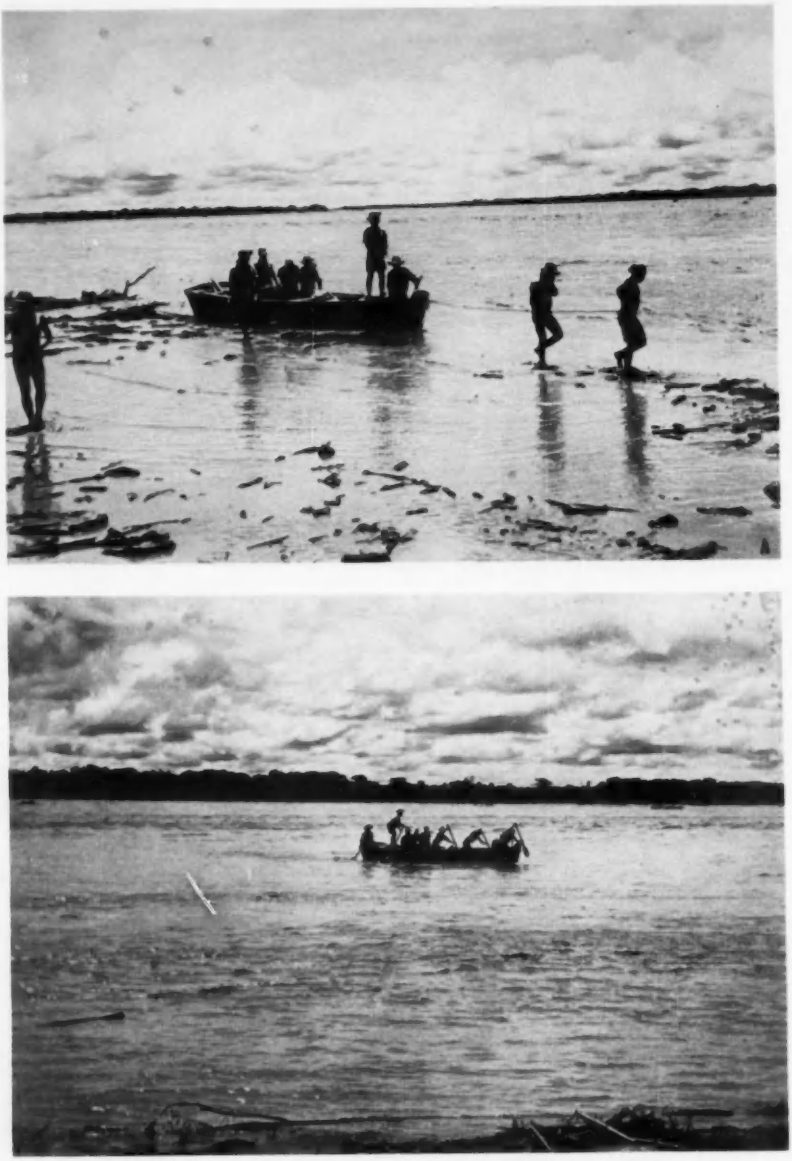

FIG. 7. Indian boat crew from settlement of Puerto Cespedes who provided passage for the medical team across the flooded Rio Grande.

ETIOLOGY

The clinical picture of jungle fever as encountered at Uruma was similar to that of many clinically nonspecific febrile diseases frequently seen in nonindigenous people engaged in clearing and settling virgin jungle lands.[5-7] Even indigenous individuals are not immune to all infectious agents latent in the jungle environment

FIG. 8. Medical team along flooded trail to Uruma colony: (a) Dr. Morris Schaeffer seated on raft bearing medical supplies. The raft is pushed by men from the Okinawan colony; (b) expedition party on horseback along flooded trails; (c) at many places along the route the horses were required to swim because of the depth of the water.

FIG. 9. Views of the West Barracks at the Uruma colony showing border of virgin jungle in background.

surrounding them, and they too may become victims of endemic infections, surviving in natural reservoirs in their rain forests, when they enter into intensive contact with a disturbed ecological environment such as occurs during clearing of jungle.[5-9] Fleas, mites, ticks and mosquitoes (particularly those from the high canopy foliage) and other arthropod vectors which do not usually

FIG. 10. Okinawan children outside of barracks building at the Uruma colony

FIG. 11. (a) Colonists washing clothes near well; (b) Penned pigs at Uruma colony with cultivated fields in background.

attack man may, under these circumstances, accept a human host. Contaminated primitive water sources for drinking and bathing and even such possible oddities as air-borne fungi in old tree trunks or previously undisturbed caves are all added hazards to such pioneering.

As the Causeys have shown near Belem, in Brazil, near the mouth of the Amazon, an epi-

FIG. 12. Collecting blood specimens at the Uruma colony

demic of nondescript fevers in a group of laborers clearing such virgin rain forest may be caused by several different neurotropic viruses rather than by one agent.[9] In many parts of the tropics most such cases of pyrexia are diagnosed as malaria without proving the diagnosis. Similarly, in infants and children in regions of primitive medical practice, a wide variety of acute infections are dismissed as "pneumonia," although there are usually no laboratory data to support such a diagnosis or to suggest the etiology of the illness. However, even with the aid of a well-equipped clinical laboratory, specific etiological diagnoses are often difficult or impossible to establish. Clinical observation and clinical laboratory study, therefore, could hardly be expected to unravel the etiology of the jungle fevers at Uruma.

It should be noted that the colonists had settled in virgin jungle near known areas of endemic yellow fever. They had not been vaccinated against yellow fever before leaving Okinawa and were engaged in clearing jungle until on November 5, 1954, approximately 3 months after their arrival, when they were all vaccinated with type 17D yellow fever vaccine. The vaccine was carried overland from Santa Cruz and was without refrigeration for approximately 48 hours. Therefore, its immunizing potency could be questioned. For these reasons and because yellow fever is known to be endemic in the Santa Cruz area, the possibility of yellow fever, modified by partial immunization, was seriously considered at first. However, all doubts were dispelled after laboratory tests on vials of the vaccine brought back to the United States for reassay proved them still potent. Moreover, there were no significant differences in neutralization and hemagglutination-inhibiting yellow-fever antibody patterns between colonists who did not become ill and those who had recently recovered from the illness.

During the visit of the United States team to Uruma in March, 1955, mice, guinea pigs and rabbits were inoculated intraperitoneally with blood from three acutely ill patients. Rabbits so inoculated remained well for 30 days. Accidental death of the other animals within a few days following inoculation precluded continuous observation. However, further animal passage of their tissues was not productive of any infectious agent. Cultures of the same blood specimens on Fletch-

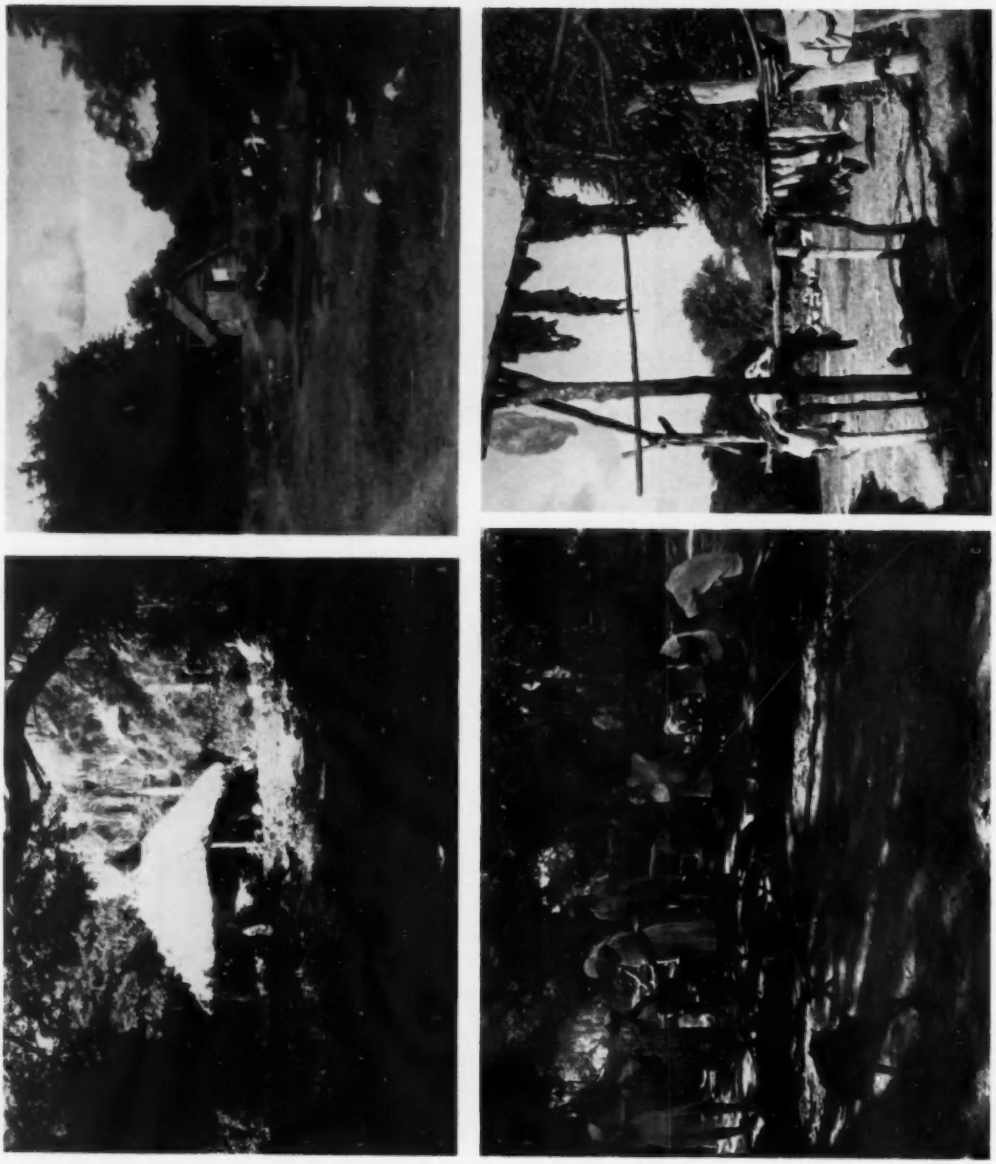

FIG. 13

FIG. 14. Puerto Cespedes residents who are mixed bloods of the Indian and white races. (a & b) children; (c) mother and child at jungle ox cart which is the only vehicle used on the flooded jungle tracks.

FIG. 13. Puerto Cespedes settlement of indigenous Bolivians on east bank of Rio Grande: (a) thatched roof dwelling; (b) bathing pool before dwelling; (c) outdoor kitchen at Puerto Cespedes; (d) meat and hides drying in sun.

er's and Schüffner's media were negative for leptospires. Clotted blood, and blood inoculated into HeLa and MB-cell tissue-culture tubes from these three acutely ill patients, were brought back to the United States where, from both the serum and the clotted blood and from the MB-cell culture tube inoculated with fresh blood of one of the three patients, a new virus, pathogenic for suckling mice by the intracerebral route of inoculation, was isolated. This virus was obtained from patient Y. S. (Case 1), of the two typical cases presented above, who was seen by us on her first day of fever and bled at that time. This virus has been shown to be a new agent apparently belonging to Casals' Group A arthropod-borne viruses.[7] It differs from all known enteric, respiratory and neurotropic agents, but serologically is related to Semliki Forest virus. Its complete characterization is presented in the second paper of this series.[4] The patient from whom the virus was isolated developed, during convalescence, a remarkably high level of neutralizing antibody to Uruma virus. Thus, a serum specimen collected on the first day of her disease had a neutralization index for the Uruma virus of less than 8, while a specimen of serum collected about 7 weeks from the onset had a neutralization index of over 250,000. A second convalescent specimen collected 4 months after her disease revealed the persistence of specific neutralizing antibody at a high level.

The Uruma virus is thus the established cause of disease in one of the cases of jungle fever; and this was clinically a fairly typical case of the disease. Subsequent to the recovery of the virus from blood of this first patient, the virus was similarly isolated from the stored, frozen blood clot of another of the three patients seen in the acute stage of illness in March, 1955. An additional three of 38 investigated convalescent serum specimens had neutralizing indices to the Uruma virus of such magnitude that it appears unquestionable that the subjects were recently infected with this agent. Thus, Uruma virus was probably the cause of disease in these cases. Furthermore, two of the 38 studied cases had neutralizing indices in the lower range of questionable significance, but these cases might also have been the result of infection with this virus or some serologically related agent.

The remaining sera examined lacked significant antibody titers to the Uruma virus, and in the case of these patients it is very unlikely that this virus, or one serologically related, played any role at all in the etiology of their illness. We can thus conclude that Uruma fever probably accounted for 10% of the cases in this outbreak and that at most 15% of the cases could conceivably have been the result of infection with this agent. The remaining cases must have been caused by a different agent or agents. It is thus apparent that in the Uruma epidemic of jungle fever we were dealing with pyrexias caused by more than one microorganism. Furthermore, it seems unlikely that any significant number of the cases was caused by any of the numerous agents which are listed in Table 4. The majority of cases of jungle fever thus remain fevers of unknown origin, caused by as-yet-unknown microorganisms.

Serum specimens were collected by the field party from approximately 100 patients at varying states of convalescence and from approximately 30 colonists who had remained well throughout the epidemic. The serological studies which have been completed to date are summarized in Table 4. The results of agglutination-lysis tests on convalescent sera for 17 sero-types of leptospires, complement-fixation tests for Q fever, epidemic typhus, murine typhus, Rocky Mountain spotted fever, rickettsialpox, St. Louis encephalitis, Western equine encephalomyelitis, Eastern equine encephalomyelitis, coccidioidomycosis, blastomycosis and histoplasmosis antibodies and neutralization tests for encephalomyocarditis (AMSGS strain) and Venezuelan equine encephalomyelitis were essentially negative and provided no clue to the epidemic. Hemagglutination-inhibition tests for Venezuelan equine encephalomyelitis and for Semliki Forest virus antibody were negative while similar tests for Ilheus virus antibody, although largely positive, revealed no significant differences between a group of sera from convalescent patients and colonists who had not been ill. Thus, none of the agents mentioned above appeared to be the cause of the epidemic or of any significant number of cases of the illness in patients. The presence of group B antibody in the sera from many patients, as well as in sera from many colonists who had no clinical diseases, might be interpreted as evidence of cases of inapparent infection. However, such antibody in the titers encountered would be expected in Okinawan populaces who have resided in regions where Japanese B encephalitis was prevalent and who had, furthermore, received yellow fever vaccine.

The field team collected an additional 66 serum

TABLE 4

Results of serological investigations of serum specimens from Okinawan patients convalescent from jungle fever

Type of test	No. of sera tested	No. with diagnostically signif. titers	Remarks
Complement fixation			
Rickettsioses:			
Q Fever	32	0	19 colonists who had not been ill, also were all negative.
RMSF	5	0	
Rickettsialpox	13	0	
Epidemic typhus	24	0	3 positive at 1:20. Of 19 colonists who had not been ill, one had a titer of 1:10 and one of 1:5.
Murine typhus	32	0	1 positive at 1:16, 1 positive at 1:10, 2 at 1:5. Of 19 colonists who had not been ill, 1 had a titer of 1:20.
Psittacosis	13	0	1 positive at 1:4.
Lymphogranuloma venereum	13	0	1 positive at 1:4.
Mumps	13	0	Both soluble and viral antigens used. 4 sera positive at 1:4 with viral antigen.
Influenza A	13	0	Many sera gave 1:4 and one a 1:8 titer with influenza A or B.
Influenza B	13	0	
Neurotropic viruses:			
St. Louis encephalitis	13	0	
Eastern equine encephalomyelitis	13	0	
Western equine encephalomyelitis	13	0	
Lymphocytic choriomeningitis	13	0	
Toxoplasmosis	7	0	
Mycoses:			
Blastomycosis	12	0	
Histoplasmosis	12	0	
Coccidioidomycosis	12	0	
Agglutination			
Leptospiral agglutination-lysis	19	0	4 sera positive at 1:100 to *L. icterohemorragiae*, *L. javanica*, *L. hebdomadis*, and *L. autumnalis*, respectively. 1 serum positive to *L. ballum*, *L. grippotyphosa*, *L. javanica*, *L. alexi* at 1:100 and to *L. pyrogenes* at 1:400. Of 11 colonists who had not been ill all the tests for leptospirosis were negative.
Histoplasmin collodion agglutination	5	0	
Plague (purified capsular antigen of *P. pestis*)	19	0	2 sera positive at 1:40.
Hemagglutination Inhibition			
Venezuelan equine encephalomyelitis	10	0	Of 10 colonists who had not been ill none had antibody.
Semliki Forest virus	10	0	

TABLE 4—*Continued*

Type of test	No. of sera tested	No. with diagnostically signif. titers	Remarks
Yellow fever	10	7	Of 10 colonists who had not been ill 4 had positive tests. Titers were low, ranging from 2 to 160.
Ilheus	10	9	Of 10 colonists who had not been ill 7 had antibodies. Titers ranged from 20 to over 640.
Neutralization			
Yellow fever	10	6	Results of a comparison, by the yellow fever mouse-protection test, of 10 convalescent sera with 10 sera from colonists who had not been ill, were as follows:
			No protection / Equivocal / Protection
			Convalescent 4 / 5 / 1
			Not ill 2 / 2 / 6
Encephalomyocarditis	14	0	3 sera with equivocal neutralization indices of 1.1, 1.5 and 1.5, respectively.
Venezuelan equine encephalomyelitis	19	0	
Ilheus virus	4	0	

specimens from children and adults of all ages in the small isolated community of Puerto Cespedes on the Rio Grande, the nearest settlement of indigenous Bolivians to the Uruma colonists. Since none of the subjects bled had had travel experience out of the region, any antibodies found must have had their source in this jungle region west of the Brazilian Matto Grosso. These sera from Puerto Cespedes have been used together with sera from the Peruvian Amazon shore communities in a serological survey of the Upper Amazon Drainage which has been reported elsewhere.[3] However, the results of this survey indicated that Uruma virus was active in this locality and that at least 38% of the individuals had had previous experience with this virus, or with an immunologically closely related agent.

From the above data we may conclude that some of the cases of jungle fever at Uruma were caused by this newly isolated virus. Uruma virus is an agent pathogenic for man and productive of a febrile disease without pathognomonic clinical signs which we designate as Uruma fever. This virus may have accounted for 10–15% of the cases in the epidemic. From serological studies on indigenous inhabitants of this jungle region it is apparent that the Uruma virus is endemic here. In the second paper of this series, dealing with the characterization of Uruma virus, it will be shown that this agent belongs to the serological subgroup of the Type A arthropod-borne viruses, which includes the strains of Semliki Forest, Mayaro, Chikungunya and Sindbis viruses. Thus, the closely related Uruma and Mayaro strains of virus cause febrile disease in man over a wide, jungle-covered region of South America and arthropod-borne viruses of this serological subgroup of Type A are significant causes of the disease in man over a large portion of the tropical forested regions of the world.[4, 8, 10-13]

DISCUSSION

The epidemic of jungle fever which caused the failure of the Uruma colony thus remains only partially solved etiologically. The isolation of a viral agent responsible for over 10% of the cases was a fortuitous success for an epidemiological expedition paying a brief and cursory visit to the stricken colony. From the study of this epidemic among the Okinawan pioneers in a remote, tropical, rain forest, under conditions of primitive medical and laboratory facilities, it may be concluded that it is impossible to determine how many etiologically different pyrexias actually

abounded to constitute the entire epidemic. Infectious agents may have been prevalent in the early months of the epidemic which differed from those in the latter months, although there are no clinical data which clearly indicate a diverse etiology. However, variations in the respiratory symptoms, lymphadenopathy and in the extent and appearance of a rash all might suggest the possibility of multiple disease entities.

Any nonindigenous population engaged in clearing and putting into agricultural productivity a region of virginal tropical rain forest may be expected to suffer from a high incidence of infectious diseases latent in such environment. These "jungle fevers" are probably transmitted by arthropod vectors and may be clinically nondescript and undifferentiable from infections with respiratory and enteric pathogens which are likely to occur under the conditions of transient overcrowding and poor sanitation accompanying a tropical pioneering venture. To predict in advance what endemic viral agents may threaten a group of colonists would involve thorough knowledge of the potential human pathogens which are endemic in the region and of the complex ecological cycles involving mammals, birds and arthropod vectors which may exist. Such information is seldom available for a region being opened to human habitation. Therefore, one could expect to cope with any medical emergencies which might develop only if intensive and continual medical and epidemiological surveillance of the colonizing community were available. In addition to this, ecological studies involving a thorough entomological study of potential arthropod vectors should precede and accompany the early stages of jungle clearing and settlement. Such study, supported by a well-equipped microbiological laboratory, could bring the assistance of current medical knowledge to ventures in jungle settlement, but even such medical support cannot be expected, with certainty, to prevent the occurrence of fevers of unknown origin, the etiology of which will be found only in retrospect in the laboratory or remain undetermined, and which, in some cases, will be a new viral agent or agents.

SUMMARY

An epidemic of jungle fever which involved almost one-half of 400 Okinawan pioneers who were attempting to settle in the jungle rain-forest of the Bolivian lowlands adjacent to the Brazilian Matto Grosso is described. Fifteen of the cases ended fatally.

A new virus, one of the Group A arthropod-borne viruses, serologically related to Semliki Forest virus, has been isolated from the blood of two of the patients. This, the Uruma virus, was responsible for 10 to 15% of the cases of jungle fever; the etiology of the remaining cases remains undetermined. Serological survey of indigenous communities reveals that this virus or a serologically related agent is endemic to the region. The Semliki Forest-Uruma-Mayaro-Chikungunja serological group of agents appears to be a world-circling group of tropical Group A arthropod-borne viruses pathogenic for man.

The problem of jungle fever, or pyrexias of unknown origin, in similar attempts of nonindigenous populaces to clear and settle in tropical jungle, and the problem of multiple etiology of any epidemic that develops among them, are discussed.

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BOOKS RECEIVED

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Disease and Population Pressure in the Tropics—Two Lectures at University College, Ibadan, Nigeria, by T. H. Davey, visiting Nuffield Professor, 1958. 22 pages. Ibadan, Nigeria, Ibadan University Press, 1958. 2/-.*

The Ecology of Human Disease, by Jacques M. May, M.D., with foreword by Felix Marti-Ibañez, M.D. 327 + xxiv pages with maps. New York, MD Publications, Inc., 1958. $7.50.

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Treatment of Lung Cavities and Endobronchial Tuberculosis, by Beryl E. Barsby, M.D., M.R.C.P. 147 pages, ill. Edinburgh and London, E. & S. Livingstone Ltd., (Williams & Wilkins Co., Baltimore, exclusive U. S. agents), 1959. $4.75.*

Parasitology (Protozoology and Helminthology), by K. D. Chatterjee, M.D. 2nd edition, 188 pages, ill. Calcutta, by author, 1959. Rs. 17.50.

Seminar on the Susceptibility of Insects to Insecticides—Final Report, by various authors. 335 pages. Washington, D. C., Pan American Health Organization, 1959.

BOOK REVIEWS

Leptospirosis in Man and Animals, by J. M. Alston, M.D., F.R.C.P. Edin., J. C. Broom, O.B.E., M.D., and C. J. A. Doughty, B.A., with foreword by Sir Samuel Bedson, M.D., D.Sc., F.R.C.P., F.R.S., 367 pages, illustrated. Edinburgh & London, E. & S. Livingstone, Ltd. (Williams & Wilkins exclusive U. S. agents), 1958, $8.50.

This is an exhaustive presentation and might well be titled "A Treatise on Leptospirology," (to coin a term). It is developed on a chronological basis, which makes for easier understanding and clarification of the mass of data presented. The style of writing is clear and interesting. It presents every important aspect of the subject in detail, with documentation on every point. There is a list of about 850 references, an Index of Authors and Sources and an Index of Subjects. The book is illustrated with interesting protraits and with photomicrographs and electronographs of varying degrees of clarity. The four sections deal with, first: Morphology, Classification, Physiology and Distribution of Leptospires (64 pages); second, Leptospirosis in Man (71 pages); third, Leptospirosis in Animals (39 pages); fourth, Leptospirosis in Regions Most Affected (23 pages). An Appendix presents methods for cultivation and serological study. The authorship of the book

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Referred for review to "Tropical Medicine and Hygiene News," Editor: Dr. Louis J. Oliver, Laboratory of Tropical Diseases, National Institutes of Health, Bethesda 14, Maryland; Published by **The American Society of Tropical Medicine and Hygiene.**

guarantees authenticity and justifies the viewpoint. However, all experts rarely (or never?) agree though they may be hard pressed to find material for debate. For example, one might safely believe that Walter Reed proved the viral nature of the etiological agent of yellow fever, that Stokes, Bauer and Hudson re-established the fact on more extensive data, and that Sawyer and his coworkers demonstrated the viral nature of the disease in Brazil, using Noguchi's leptospires as control material. No one interested in any aspect of leptospirosis can neglect this valuable work.

M. FROBISHER

Quadriennial Report of the Director of the Pan American Sanitary Bureau, Regional Office of the World Health Organization, 1954–1957, and the 1957 Annual Report, by FRED L. SOPER, M.D., Director, PASB. 238 pages, illustrated. Washington, D. C., Pan American Sanitary Bureau, 1958.

This impressive exposition of the growth and activities of the Bureau over the past 4 years deserves more attention than is usually accorded to institutional reports. Growth bears a relation to success, and one notes a 24 per cent increase in budget (now about 4 million dollars) over 1956, and a 17 per cent increase in staff, now numbering over 600, two-thirds of whom are in the field. The novelty of the report is the concept of eradication versus control of certain communicable diseases. Most of the Director's introductory essay is devoted to an interesting and optimistic discussion of eradication, first proposed by Brazil in 1947 in the form of a hemispheral campaign for the elimination of *Aedes aegypti*, and soon extended to malaria, yaws, and smallpox, all four of which are in full retreat and may be on the way out. There is a good chance that these can be eliminated from the Western Hemisphere within a reasonable time and at a cost not difficult to justify when one considers the enormous damage they have wrought over the centuries. It can be predicted that the concept will, with more deliberate speed, eventually include TB and VD, those two firmly intrenched enemies which, considered not long ago to be medico-social problems insoluble except through social evolution, have now been transformed by modern therapeutic measures into questions of funds and personnel. This cannot be said of two other great problems of Latin America, malnutrition and the zoonoses, which require more knowledge in technics than we now possess before we can hope to affect them materially.

While communicable diseases are still the most important health problem of the Region, PASB maintains a balanced program of technical aid to central health services, education and training of personnel and pilot projects in integrated programs of health administration. The report leaves one with the impression that a new kind of solidarity has been brought about by a common concern about health among a group of nations not noted for their cooperative spirit. Probably the concept of hemispheral eradication of diseases has contributed to this greatly, since success in such an enterprise makes indispensable an action across frontiers of the most peaceful and mutually beneficial kind.

L. W. HACKETT

International Standards for Drinking-Water, by World Health Organization. 152 pages. Geneva, World Health Organization, 1958. (Columbia University Press, U. S. Distributors). $4.00.

This volume represents the work of an international group of experts over some years, finally summarized in a meeting at Geneva in 1956. It deals only with health aspects of public water supplies for domestic use. The methods given are carefully selected and are based largely on procedures described in "The Bacteriological Examination of Water Supplies" of the Ministry of Health and the Ministry of Housing and Local Government for England and Wales, and "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes," 10th edition, of the American Public Health Association, with modifications directed mainly toward simplification. The coliform group is used as the principal indicator organism, and its determination follows the familiar "presumptive," "confirmed" and "completed" tests. Azide and tellurite methods are given for fecal streptococci and a boiled-milk procedure for *Cl. perfringens*, and their significance is discussed. Numbers are expressed in MPN based on tables revised by Swaroop. IMViC procedures are given for *E. coli*.

Results of chemical procedures are to be expressed in milliequivalents/L, ppm is to be abandoned. Chemical components are to be expressed in ions. Color is related to the Pt-Co scale. Chemical methods comprise As (Gutzeit), fluoride (Scott-Sanchis), BOD (Alsterberg modification of the Winkler procedure), chlorides (Mohr), Cu (diethyldithiocarbamate) and so on, including standard tests for Cr, ^{-}CN, $^{-}NO_3Fe$, Mn, Zn, pH, albuminoid N, $^{-}NO_2$, etc. The memberships of the study groups are given, and a list of 29 pertinent references is included

This manual has a peculiar value in that it presents an internationally selected and internationally acceptable methodology to all countries interested in maintaining a water supply commensurate with international travel, trade and social intercourse in a day when a Canadian

merchant (so inclined) may have his (early) breakfast in a hotel in Mexico City with a German manufacturer and his (late) dinner with an Arabian buyer the same evening in Baghdad.

M. FROBISHER

World Health Organization Annual Epidemiological and Vital Statistics. 1955. 699 pages. Geneva, W.H.O., 1958. $12.00, £3, Sw. Fr. 36.

This is the 8th annual volume of vital and health statistics published by the World Health Organization. The report is divided into three main parts: first, vital statistics; second, statistics of cases and deaths from notifiable diseases; and third, statistics of health personnel, hospital establishments and vaccinations. In all three parts the statistics are given for the individual countries of the world for which figures are available. There are two indexes, by country and by subject.

Part I includes population figures by sex and age. These figures are less detailed than those given in the Demographic Year Book published by the United Nations but are adequate for the interpretations of the present report. The data presented include natality, general mortality, infant and neonatal mortality, late fetal mortality, and natural increase. Numbers as well as rates are given, by cause of death, sex, and age. A greater detail about malignant deaths is given than in earlier reports.

Part II, which may be of greatest interest to the readers of this Journal, presents for the first time cases of communicable diseases, by sex and age, as well as deaths.

Part III, which was initiated in 1954, gives information on the number of different types of health personnel, number of hospitals and beds by type, and vaccinations against specified diseases.

The two page Introduction is one of the most important parts of this volume. In addition to indicating the contents of the tables, it gives a brief discussion of the sources of the data, and a warning as to their comparability. The information has been taken from official sources and checked by the national administrations. However, as is pointed out, only about 42% of the world's population is subject to a system of civil registration for births and only 33% for deaths. The vital statistics necessarily vary in quality from country to country and frequently the quality is difficult to assess. An effort has been made to omit fallacious demographic data but no selection has been made on communicable disease data. Justification is given for this on the ground that within a country the completeness may be about the same from year to year and from season to season, and the report purposely omits population data in these tables to discourage international comparisons which would be unsound.

The value of a report that brings together such a wide variety of data from official sources hardly needs emphasis. This represents a most welcome service performed by the statisticians of WHO. The warning in the Introduction relative to the over-interpretation of figures of poor or of unknown quality needs a great deal of emphasis. Particularly with regard to communicable diseases, one must bear in mind that reporting may be extremely poor even in countries with well-developed health departments and statistical systems. Furthermore, definitions of even such objective and seemingly countable items as hospitals, beds, and doctors are very difficult to formulate and probably vary widely from one area to another.

Users of the volume should read and reread the Introduction so that the ground-rules for assembling the data will be clear and the inherent limitations of the data, fully appreciated. The inclusion of this statement, as well as the tables themselves, will be valued by the health professions.

MARGARET MERRELL

Immunization Information for International Travel, by U. S. Department of Health, Education and Welfare. June, 1958 revision, 71 pages. Washington, D. C., Government Printing Office, 1958. $.30.

As stated in the preface of this publication, "Americans travel abroad more than the nationals of any other country—today more than any other time in history." For the convenience of those planning to travel, the United States Public Health Service has prepared this excellent booklet which presents in a concise way the immunizations required for traveling abroad. Under the International Sanitary Regulations of the World Health Organization, the health authorities at ports of arrival may, under certain conditions, apply sanitary measures to and require certificates of vaccination from travelers to prevent the spread of the six quarantinable diseases. In this publication, the immunizations requested by 200 countries and territories are listed by continents and by alphabetical order within the continent for easy reference. Information is also given on immunizations other than those enforceable under the international agreements, which are recommended —as an added precaution—either by the countries themselves or by the United States Public Health Service. Wisely so, a general suggestion is made that all travelers be immunized against typhoid and paratyphoid fever, tetanus and poliomyelitis

in view of the high prevalence of these diseases in many areas of the world. Yellow fever immunizations, required by many countries, are the only vaccinations which are not internationally accepted when given by private physicians and must be secured from approved yellow fever vaccination centers. A list of 69 of these centers, distributed throughout the United States and its territories, is given. Finally, detailed immunization requirements for entering the United States, its territories and possessions, as well as the regulations for the importation or re-entry of pets, complete the information embodied in this booklet.

In summary, this is a most useful publication which will be welcomed by the ever-increasing number of Americans who are traveling abroad.

MARIO PIZZI

Insecticide Resistance in Arthropods, by A. W. A. BROWN. World Health Organization, Monograph Series No. 38. 240 pp., illustrated with figures and tables. WHO, Palais des Nations, Geneva, 1958. Price $5.00, U. S. A.

One of the most significant advances of recent years in the field of public health has been the discovery of the usefulness of DDT and other chlorinated hydrocarbons in the control of disease-bearing arthropods. As a result, it is now possible to organize programs against malaria and certain other diseases with eradication rather than control as the objective. However, in the past few years, almost 40 species of insects of public health importance have developed resistance to one or more of these compounds. In this book, Dr. Brown has presented a summary of all published and some unpublished information concerning insecticide resistance among these arthropods.

Evidence points to DDT resistance, due to DDT-dehydrochlorinase, as being controlled in most cases by a single gene. In other insects, several genes appear to be involved. The mechanism and genetics of resistance to the cyclodiene compounds are not so clear, although in some instances this too appears to be monofactorial. BHC-resistant flies may not contain DDT-dehydrochlorinase, but nevertheless are able to convert BHC into pentachlorocyclohexene. An increase in vigor may permit a strain to tolerate larger doses of an insecticide; this is known as vigor tolerance and appears to be due to multiple factors.

Measures to counteract the development of resistance include substitute insecticides, sanitation, and periodic checking for resistance so that substitution of a different chemical may be made before a high level of resistance has developed. Biological factors affecting susceptibility are stage of development, age, sex, nutrition, temperature, and seasons.

These and other phenomena associated with insecticide resistance are presented in this book. The completeness of the coverage is indicated by the titles of the chapters: Nature and characterization of resistance, Taxonomic groups characteristically vectors to man, The housefly, and Taxonomic groups characteristically not vectors to man. The resistant vector species are found among the Muscoid Diptera, Psychodidae (but not *Phlebotomus*), Culicidae, lice, and fleas; non-vector species include various flies, bedbugs, cockroaches, and ticks. In the chapter on *Musca domestica*, the genetics, physiology, cross resistance, and other aspects are presented in greater detail.

This is a very broad subject, and in a book of 240 pages, conciseness and clarity are essential. Dr. Brown has been markedly successful in these respects in his summarization of the literature. The reader who wishes to pursue further any given topic will find a complete bibliography in 27 pages of references. This is a timely and important contribution to public health, and is recommended to readers of this journal, as well as to all other workers in public health and related fields.

LLOYD E. ROZEBOOM

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and arthropod-borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted articles which raise no question of scientific priority but for which immediate publication seems important will be inserted in the next available issue of the journal if the author or sponsoring institution will pay the cost, estimate of which may be obtained from the editor. The insertion of such articles does not affect publication of other manuscripts awaiting their turn, since the inserted articles constitute additional pages in the volume without additional cost to the subscriber.

Publication will be facilitated if the following suggestions are followed:

1. *Manuscripts* should be typewritten in *English* using *double spacing* on *one side* only of 8½ x 11 inch paper and submitted *in duplicate*. Double-spacing should be used throughout the manuscript including the list of references, footnotes, and quotations.

2. The author should supply, on a separate sheet, a short title not over 50 letters for use as a running head. Also on separate sheets type (a) the authors' names and affiliations; (b) tabular matter; (c) case histories; (d) quotations; (e) formulas and other subsidiary matter in the text; (f) footnotes; (g) references; (h) legends for illustrations; (i) displayed formulas occupying more than 1 line of type, even if using the same size type as the main text.

3. Legends should be separate and not attached to, or written on, the illustration copy.

4. Positions for tables and figures in the text may be indicated on the margin of the text pages.

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6. *Tables* should be typed on separate sheets and be serially numbered in Arabic numerals.

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